

2010

Investigating the control of *Listeria monocytogenes* on uncured, no-nitrate-or-nitrite-added meat products

Kohl Danielle Schrader
Iowa State University

Follow this and additional works at: <https://lib.dr.iastate.edu/etd>



Part of the [Animal Sciences Commons](#)

Recommended Citation

Schrader, Kohl Danielle, "Investigating the control of *Listeria monocytogenes* on uncured, no-nitrate-or-nitrite-added meat products" (2010). *Graduate Theses and Dissertations*. 11551.
<https://lib.dr.iastate.edu/etd/11551>

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

**Investigating the control of *Listeria monocytogenes* on uncured, no-nitrate-
or-nitrite-added meat products**

by

Kohl Danielle Schrader

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Meat Science

Program of Study Committee:
Joseph C. Cordray, Major Professor
Joseph G. Sebranek
Dennis G. Olson
Kenneth J. Prusa
Aubrey F. Mendonca

Iowa State University

Ames, Iowa

2010

Copyright © Kohl Danielle Schrader, 2010. All rights reserved.

Dedication

To my mentor and friend, Klaus-Peter Kreibig. Thank you for all of your lessons around the bowl chopper, stuffer and smokehouse. You gave me an education that no book or classroom setting could ever provide. I will always cherish our friendship and the knowledge that I have gained from you over the years. I promise that I will never forget “to act, think and be like the sausage.”

TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION	1
Dissertation Organization	4
CHAPTER 2. LITERATURE REVIEW	5
I. The History of Nitrite Usage in Processed Meats	5
II. Regulations in the United States with Nitrate and Nitrite	6
III. Importance of Nitrite in Processed Meat Production	10
a. Color	10
i. Pigments of Meat Color	11
ii. Meat Color Chemistry	13
iii. Fresh Meat Color	14
iv. Cured Meat Color	16
v. Cured Meat Chemistry	18
vi. Cured Meat Color Development	21
b. Flavor Development and Protection	24
c. Antimicrobial Properties	27
IV. Residual Nitrite	31
V. Nitrite and Human Health	33
a. Toxicity	33
b. Nitrosamines and Cancer	34
c. Exposure and Sources of Dietary Nitrate and Nitrite	37
VI. Uncured, No-Nitrate-or-Nitrite-Added, Natural or Organic Meat Products	39
a. Rationale	39
b. Definitions of Natural, Organic, and Uncured Meat and Poultry Products	40
c. Additional Processing Considerations	43
i. Organic Processed Meat Products	43
ii. Natural Processed Meat Products	44
d. Manufacturing Methods for Uncured, No-Nitrate-or-Nitrite-Added Meat Products	46
VII. Concerns Associated with Uncured Meat Products	49
a. <i>Listeria monocytogenes</i>	50
i. Background and History	51

ii. Presence in Foods and in the Environment	52
iii. Factors Affecting the Growth and Survival	52
iv. Foodborne Listeriosis	58
v. Incidence of Foodborne Listeriosis	59
vi. Regulations Regarding <i>Listeria monocytogenes</i> in RTE Meat Products	60
VIII. Control of <i>L. monocytogenes</i> in Uncured, No-Nitrate-or-Nitrite -Added (Natural or Organic) RTE Meat Products	62
IX. Summary of Literature	67
References	68

CHAPTER 3. INVESTIGATING THE CONTROL OF *LISTERIA* *MONOCYTOGENES* ON UNCURED, NO-NITRATE- OR-NITRITE ADDED PROCESSED MEATS

78

Abstract	78
Introduction	79
Materials and Methods	81
Results and Discussion	86
Conclusions	114
References	121

CHAPTER 4. IMPACT OF NATURAL AND “CLEAN LABEL” ANTIMICROBIALS ON THE GROWTH OF *LISTERIA* *MONOCYTOGENES* AND QUALITY ATTRIBUTES OF UNCURED, NO-NITRATE-OR-NITRITE-ADDED EMULSIFIED FRANKFURTER STYLE COOKED SAUSAGES

123

Abstract	123
Introduction	124
Materials and Methods	126
Results and Discussion	134
Conclusions	156
References	158

CHAPTER 5. GENERAL CONCLUSIONS	160
APPENDICIES	162
ACKNOWLEDGEMENTS	178

CHAPTER 1. GENERAL INTRODUCTION

Numerous consumers have become infatuated with the concept that minimally processed; preservative free meat products marketed as natural or organic, possess a higher level of safety and are more nutritious than conventionally processed meat products. Sodium nitrite (NaNO_2) is a common non-meat ingredient found in cured processed meat products and has been under scrutiny by such consumers for decades. While the majority of processed meat formulations include sodium nitrite as a curing agent, in rare instances sodium nitrate (NaNO_3) is added during the production of specialty cured meat products. Because nitrate and nitrite are classified as chemical preservatives, they are not permitted as ingredients that are directly added to natural or organic products. Nitrite is a unique, irreplaceable ingredient that is known not only for the development of cured meat color and flavor in processed meat products, but for its vital role in the safety of processed meats, most notably against the outgrowth of *Clostridium botulinum*. Despite its proven track record for food safety and the prevention of toxin production by *C. botulinum*, processors have begun to utilize alternative methods for the production of “uncured, no-nitrate-or-nitrite-added” meat products to meet the increased demands for preservative free meat products by natural and organic consumers.

Currently there are two types of “uncured, no-nitrate-or-nitrite-added” meat products available on the marketplace. The first option available to consumers is a product that is truly uncured, where there is no intention of replacing the nitrate or nitrite. These products lack the typical pink cured meat color and traditional flavor

notes. The second option available are products that make use of alternative production methods which utilize naturally occurring nitrates and nitrites found in vegetables and sea salts to result in processed meats that demonstrate traditional color and flavor characteristics. These processes result in typical cured meat properties but have also been found to result in greater variation of these properties than that observed for conventionally cured meats.

Conventionally cured products contain a regulated amount of nitrate or nitrite, and in turn consistency in quality and safety is easily attained. Conventionally processed meat products also utilize hurdle technologies to provide safe, wholesome meat products for consumers. Hurdle technologies use combinations of ingredients and/or additional processing procedures to reduce the occurrence and outgrowth of deadly foodborne pathogens. These meat products contain a multitude of antimicrobials including nitrite, salt, phosphates, organic acids and components of natural smoke. The aforementioned hurdle technologies are vital in the production of processed meats because many products including deli meats and frankfurters are classified as ready-to-eat (RTE) and are often not reheated prior to consumption.

Such RTE products have received a great deal of attention in recent years due to the potential for post processing contamination with *Listeria monocytogenes*. *Listeria monocytogenes* (*Lm*) is a pathogenic bacterium that is commonly found in the environment and carries a high mortality rate (~30%) if the foodborne illness listeriosis occurs. Because of the high mortality rate in at-risk populations and widespread presence in the environment, healthy animals and meat processing

facilities, compliance guidelines have been established to reduce the incidence of *Lm* in RTE meat and poultry products. RTE meat and poultry products are considered adulterated if they contain *Listeria monocytogenes* or come into direct contact with a food contact surface that is contaminated with *L. monocytogenes*. *Listeria* can contaminate and grow on RTE products if they are not formulated or produced in a manner to destroy or suppress the growth of the organism. Despite this, processors are currently utilizing alternative processing procedures for minimally processed meats that significantly reduce or eliminate the hurdles available to prevent the growth of *Listeria monocytogenes* should contamination occur.

Changes in ingredients and processing procedures in the production of natural and organic processed meats are cause for examination of uncured, no-nitrate-or-nitrite-added processed meat products to determine if significant foodborne hazards exist. In order to understand how substitutions or elimination of current hurdle technologies affect the growth of *Listeria monocytogenes* on uncured, no-nitrate-or-nitrite-added meat products, challenge studies are warranted. Therefore, the first objective of this research was to determine the current level of control that commercially available uncured, no-nitrate-or-nitrite-added RTE meat products possess to suppress the growth of *Listeria monocytogenes* when compared to conventionally processed cured meat products. The second objective was to determine what interventions are needed to improve the control of *Lm* and

what ingredients can be used as anti-listerial controls during the production of minimally processed products without sacrificing natural and organic labeling claims.

Dissertation Organization

This dissertation is organized into five chapters. The first chapter is a general introduction and background on uncured, no-nitrate-or-nitrite-added meat products and *Listeria monocytogenes*. The second chapter is a general review of relevant topics pertaining to this research project while chapters three and four are manuscripts prepared using the *Journal of Food Science Style Guide*. The third chapter is a manuscript titled “Control of *Listeria monocytogenes* on uncured, no-nitrate-or-nitrite-added processed meats.” The fourth chapter is a manuscript titled “Impact of natural and clean label antimicrobials on growth of *Listeria monocytogenes* and quality attributes of uncured, no-nitrate-or-nitrite-added emulsified frankfurter-style cooked sausages.” The fifth chapter is a general summary of this research.

CHAPTER 2. LITERATURE REVIEW

I. The History of Nitrite Usage in Processed Meats

Ancient Preservation Techniques

Early processed meat products were produced with one purpose in mind: their preservation for use in times of scarcity. Ancient civilizations recognized that cuts of meat could be preserved by treating them with a salt solution or packing them in dry salt (Pegg and Shahidi 2000). While salting was successful in controlling spoilage and extending the shelf-life of meat, high concentrations often lead to an unattractive product that was grey in appearance. As a result, a demand for certain salts increased when it was discovered that salts from particular regions gave meat products a desirable pink color and distinct flavor. It is now understood and well accepted that nitrate impurities in natural salt (saltpeter) were responsible for the attractive color and eventually led to the discovery of modern day meat curing and the deliberate addition of saltpeter (MacDougall and others 1975; Rust 1977; Sebranek 1978; Pierson and Smooth 1982; Price and Schweigert 1987). As the art of salt preservation progressed, the term “meat curing” was eventually defined as the addition of salt, sugar, spices, saltpeter (nitrate) or nitrite to meat for its preservation and flavor enhancement (Townsend and Olson 1987).

While the origin of meat curing and usage of saltpeter (potassium nitrate) has been lost in antiquity, researchers later discovered that it was nitrite which was the

active form of the curing compound. After the discovery that nitrite accumulated in products to which saltpeter or nitrate had been added, E. Polenske determined in 1891 that nitrate was converted to nitrite by bacterial action (reported in Sebranek 1978; Townsend and Olson 1987). Shortly thereafter, Kisskalt and Lehmann demonstrated in 1899 that the typical color of cured meats was due to nitrite and not to nitrate (Pegg and Shahidi 2000). The real breakthrough however, came in 1901 when the mechanism for cured color development was described as a combination of nitric oxide with meat pigments by J. Haldane (Sebranek 1978). Hoagland confirmed Haldane's findings in 1908 and his later studies in 1914 revealed that saltpeter (potassium nitrate) was an inactive compound that must first be reduced to nitrite to allow for nitric oxide production and subsequent cured color development. This scientific knowledge led to the direct use of nitrite instead of nitrate in the production of processed meat products (Pegg and Shahidi 2000).

II. Regulations in the United States with Nitrate and Nitrite

After the realization that nitrite was the active curing ingredient scientists focused their research on the direct addition of nitrite instead of nitrate. In 1923, the United States Department of Agriculture (USDA) allowed for experiments to determine what minimum levels of nitrite were required to provide consistent cured color and prevent spoilage in cured meat products. Results showed that small quantities (one-fourth of an ounce to 100 pounds of meat) of nitrite could result in successful curing of meat products. In 1925, the direct use of nitrite was approved

for red meat products due to its importance as a coloring agent (Bacus 1984) and was limited to 200 parts per million (ppm) in the finished product.

Currently, nitrite (as sodium or potassium nitrite) is added to a wide variety of cured meat products. The direct addition of nitrate is rare and is typically utilized in the manufacture of specialty products that require long production times (i.e. fermented sausages and dry-cured meat products). In these products, nitrate serves as a reservoir for the production of nitrite through bacterial reduction. If nitrate is used as a curing agent, the conversion (reduction) of nitrate to nitrite by bacteria is a critical step for the development of cured color. Conversion of nitrate to nitrite in cured meat products is dependent upon several environmental conditions and is difficult to control. As a result, a gradual shift occurred in the meat industry and processors transitioned from the addition of nitrate to the direct addition of nitrite in cured meat products. The predominate use of nitrite as a curing agent in cured meat and poultry products in the United States can be attributed to the inconsistent control associated with the reduction of nitrate to nitrite, coupled with the fact that most processors today demand faster curing methods than what is required for successful conversion of nitrate to nitrite (USDA 1995).

As the curing process was better understood and advances in processing technologies were available to meat processors, limits for nitrate and nitrite were further emphasized by the USDA in 1970. Limits for curing agents are currently established on a green (raw) meat basis for comminuted and dry cured products; while limits are based on a total formulation/brine weight for immersion cured,

massaged or pumped cured meats. Table 2.1 lists the maximum parts per million for each of the four curing agents permitted in meat and poultry products and are based on the curing method used during the production process.

Curing Agent	Curing Methods			
	Immersion Cured	Massaged or Pumped	Comminuted	Dry Cured
Sodium Nitrite	200	200	156	625
Potassium Nitrite	200	200	156	625
Sodium Nitrate	700	700	1718	2187
Potassium Nitrate	700	700	1718	2187

Table 2.1. Maximum limits for ingoing nitrate and nitrite for cured meat and poultry products. (**does not include bacon) Reported on parts per million basis. This reprint is from USDA-FSIS Processing Calculations Inspector's Handbook (FSIS Directive 7620.3).

Maximum allowable limits vary among curing methods as processing techniques differ in the efficiency with which the curing agent is brought in contact with the meat and/or poultry product (USDA 1995). Comminuted products require less nitrite for color development, because the chopping or emulsification process increases the available surface area and increases the distribution of nitrite in comparison to dry-cured, immersion cured, massaged or pumped products. Either sodium or potassium forms of nitrite and nitrate are permitted, with the exception of bacon where the use of nitrate is prohibited. Combinations of nitrate and nitrite are allowed but careful attention must be taken when these curing methods are utilized in the production process. The use of nitrites, nitrates, or a combination must not result in more than 200ppm of nitrite, calculated as sodium nitrite, in the finished

product (USDA 1995). As a result, formulation and monitoring procedures are critical during the processing of meat and poultry products that employ combinations of curing ingredients.

As previously mentioned, the used of nitrate is not permitted for use in bacon manufacture. In addition, reduced levels of nitrite are mandatory in bacon manufacture as a direct result of concerns over nitrosamine formation when finished products are cooked at high temperatures (i.e. frying). When producing dry cured bacon, where nitrite is applied directly to the surface of the meat as part of a cure mixture, a maximum of 200ppm of sodium nitrite or an equivalent of potassium nitrite (246ppm) is permitted. If processors utilize immersion curing, massaging or pumping technologies for skin (rind) off bellies, sodium nitrite is limited to 120ppm (or 148ppm potassium nitrite).

To further complicate matters in bacon production, the maximum limit for ingoing nitrite must be adjusted if bacon is prepared from pork bellies with attached skin (rind-on). These lowered levels are required in this particular class of product due to the fact that the skin comprises ten percent of the weight of a pork belly, and will retain no cure solution or curing agent (USDA 1995). Consequently, levels must be reduced by ten percent and therefore are limited to 108ppm for pumped, massaged, immersion cured or dry cured rind-on bacon. Reduced levels of nitrite are also permitted in skinless (rind-off) bacon when used in conjunction with alternative processing procedures. Sodium and potassium nitrite are permitted at levels of 100ppm and 123ppm respectively, when used in combination with an

appropriate partial quality control program. Levels may be further reduced (40-80ppm sodium nitrite and 49-99ppm potassium nitrite) when sugar and a lactic acid starter culture are used (USDA 1995).

The United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) requires a minimum of 120ppm ingoing nitrite in all cured products that are labeled “Keep Refrigerated” unless the establishment can demonstrate safety is assured by other preservation processes including thermal processing, pH or moisture control. While minimum levels are established for products that require refrigeration, there is no regulation for minimum nitrite levels in cured products that have been processed to ensure their shelf stability (USDA 1995).

III. Importance of Nitrite in Processed Meat Production

Color

It is no secret that color plays a vital role in the consumer’s perception of the quality and safety of meat products. According to Price and Schweigert (1987) color is one of the most important characteristics of fresh and cured meats given that it is the primary attribute judged by the consumer before purchase. Meat purchasing decisions are influenced by color more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness (Mancini and Hunt 2005). The color of meat may range from the deep purplish-red of freshly cut beef to the light pinkish-gray of faded cured pork. Myoglobin is the muscle pigment which is responsible for the color changes that occur in meat

products (Rust and Olson 1973). The heme pigment component of myoglobin is the principal source of meat color and the reactions of heme pigments are important in determining the colors of both fresh and cured meat products (Fox 1966a). For that reason, it is imperative to understand the fundamental concepts of muscle and myoglobin chemistry as well as what factors will impact color and how they can be controlled throughout the production process.

Pigments of Meat Color

The color of raw meat is governed by the concentration and chemical nature of its hemoproteins or heme pigments (Pegg and Shahidi 1997). While there are a number of heme pigments in the meat system myoglobin and hemoglobin are the most abundant and fundamentally important (Pearson and Gillett 1996). Both myoglobin and hemoglobin are members of the sarcoplasmic protein class and are classified as water-soluble proteins. Hemoglobin is the primary pigment in the live animal and functions by carrying oxygen in blood to the muscle tissues. Nevertheless, after slaughter and thorough bleeding myoglobin prevails as the principal muscle pigment (Pearson and Gillett 1996). Only a very small portion of hemoglobin (20%) that is left in the muscle capillaries, arteries and veins remains in the muscle after slaughter (Fox 1966a; Romans and others 2001). When present, hemoglobin plays a small role in meat color but not to the extent of myoglobin (Price and Schweigert 1987). Because myoglobin is a water soluble protein and the most predominant heme pigment found in muscle, it is often found in the purge of a package of meat. Thus, the drip that leaks from raw steaks and other meat cuts is

not blood as commonly believed, but mainly myoglobin dissolved in the muscle cell exudate (Hunt and Hedrick 1977).

Myoglobin is produced by and found within the live muscle cells where it serves as a storage site and carrier of oxygen (Price and Schweigert 1987). The storehouse of oxygen is used in the normal biochemical processes of the muscle, which include muscle contraction and relaxation (Pegg and Shahidi 1997). The activity of muscles in the animal varies greatly and, in turn, their oxygen demands vary. Myoglobin is red in color and the concentration of myoglobin in the muscle dictates the color (Aberle and others 2001).

Various intrinsic factors are responsible for the different myoglobin concentrations found in the various muscles of the animal. The concentration of myoglobin varies according to species, being the lowest in chicken and highest in beef. In general, the myoglobin content of a muscle increases with age. In veal, myoglobin represents 1-3 mg/g, 4-10 mg/g in beef and 16-20 mg/g in beef from older animals (Price and Schweigert 1987). Anatomical location differences account for the differences in oxygen demand and in turn myoglobin concentration. This is easily observed in poultry species when comparing the muscles of the leg to that of the breast. Muscles in the leg are subjected to a higher degree of muscular activity when compared to the breast. As a result, the leg muscles would require greater oxygen supply and have a higher concentration of myoglobin.

Meat Color Chemistry

Myoglobin is a very dynamic structure containing a protein and non-protein portion. This structure can readily undergo changes in color, depending on the immediate environment (Romans and others 2001). The protein structure of myoglobin consists of 153 amino acids folded around a non-protein portion called a heme group. The heme group is composed of a porphyrin ring and contains an iron atom (Fe) as its core. (Price and Schweigert 1987; Romans and others 2001). Figure 2.1 illustrates the heme group with the six available binding sites, called ligands, for the iron (Fe) atom. Of these sites, four are used to stabilize the porphyrin ring, one is used to bind the globin (protein portion) to the heme group and the sixth site is free to interact with a number of chemical elements, for example oxygen and carbon dioxide (Romans and others 2001; Feiner 2006).

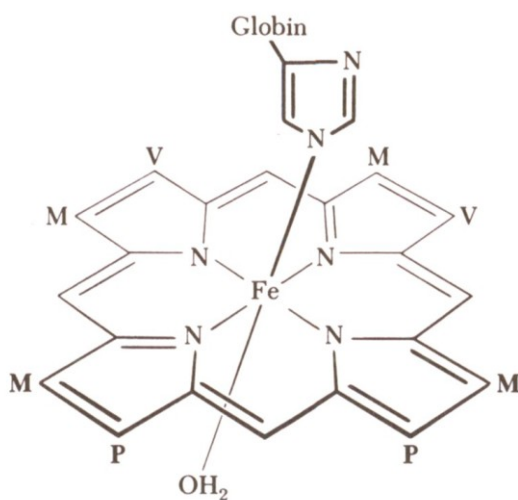


Figure 2.1. Schematic representation of the heme complex of myoglobin. (Reprinted from Price and Schweigert 1987).

It is the sixth coordination position that accounts for the function of the myoglobin molecule and its capability to reversibly bind ligands. The properties of the heme complex and color perceived by the consumer are determined by what molecule is bound at this site. Color is also dependent upon a combination of both the physical state of the protein and the oxidation state of the iron (Price and Schweigert 1987). The ability of the iron to bind ligands at the sixth position is dependent upon the oxidation state of the iron molecule, which allows for appropriate positioning of the heme group to accommodate the chemical compounds that may be present. When the iron is oxidized or in the ferric state (Fe^{3+}) it cannot combine with other molecules and form covalent complexes. Such complexes can be formed only when the iron atom is in the reduced or ferrous state (Fe^{2+}). These covalent complexes are of greatest interest because they are the bright red and pink pigments desired in fresh and cured meats respectively (Price and Schweigert 1987; Aberle and others 2001). Thus, the structure and chemistry of the iron atom are the key to understanding the changes that myoglobin undergoes (Pegg and Shahidi 1997) and subsequent color formation.

Fresh Meat Color

Meat color immediately after slaughter is a result of normal enzyme activity that continues in the muscle long after death. These enzymes utilize all of the oxygen available in the system and the pigment in uncut meat is in the reduced form (Fe^{2+}). Consequently, the iron is able to react with the water naturally occurring in the meat system (Aberle and others 2001) and the purple pigment that develops as myoglobin forms a covalent bond with water is referred to as deoxymyoglobin (Price

and Schweigert 1987). Deoxymyoglobin is commonly seen in retail cases when fresh cuts are placed in vacuum packaged bags that have removed the available oxygen in the meat system. As oxygen from the air comes into contact with exposed meat surfaces, it combines with reduced (Fe^{2+}) myoglobin pigments, and converts the purple reduced pigment to the bright red oxygenated pigment, oxymyoglobin. The development of the bright red color typically occurs within minutes of exposure to oxygen and produces the familiar “bloom” that consumers associate with freshness (Fox 1966a). The stability of oxymyoglobin depends upon a constant supply of oxygen. With time, the small layer of oxymyoglobin present on the surface of the meat propagates downward, however, the depth to which the diffusion occurs is dependent upon the enzymes involved in oxidative metabolism (Pegg and Shahidi 1997). Thus, maintaining bright red color on the surface depends on the availability of oxygen in the superficial layers of the tissues (Aberle and others 2001). Oxygen diffusion into the meat tissues is dependent upon factors such as pH, temperature, external oxygen pressure and the reducing activity of enzymes present (Pegg and Shahidi 1997).

When small quantities of oxygen are present or the rate of enzyme activity begins to slow or cease, the iron molecule becomes oxidized from the ferrous state (Fe^{2+}) to the ferric state (Fe^{3+}). As a result of oxidation, myoglobin can no longer bind oxygen, and the formation of the undesirable brown pigment, metmyoglobin occurs. Metmyoglobin formation is often problematic with regard to retail meat

display cases as consumers typically associate this brown color with a product that lacks freshness and may be unsafe.

The color cycle in fresh meats is a constant dynamic cycle of three pigments: myoglobin, oxymyoglobin and metmyoglobin. Fresh postmortem muscle possesses inherent reducing abilities that allow the reformation of myoglobin from metmyoglobin in the presence of oxygen (Faustman and others 1996). While metmyoglobin cannot absorb oxygen directly, the enzymes naturally present in meat have the capacity reduce metmyoglobin to myoglobin for subsequent oxygen absorption. Once the reduction takes place, the myoglobin, now in the reduced form (Fe^{2+}) can again bind oxygen to form the red oxymyoglobin pigment (Feiner 2006). This continuous change of pigments states results in eventual depletion of reducing enzymes causing increased metmyoglobin formation (Fox 1966a). While the bright cherry red oxymyoglobin pigment may be present on the thin surface layers, over prolonged storage metmyoglobin pigments take over and the brownish grey color dominates (Feiner 2006). In addition to the ability of meat to undergo a series of chemical reactions that ultimately dictate color, destruction or denaturation of the meat protein also plays an important role in fresh meat color. When meat is heated during cooking, an irreversible brown pigment, denatured metmyoglobin is formed.

Cured Meat Color

The distinctive pink cured color of frankfurters, ham and bacon is an important quality attribute for a consumer's acceptance and subsequent purchase of cured processed meat products. Color preferences of cured meat have been examined

and found to have a significant impact on the overall acceptance and appeal by consumer. Investigations conducted by DuBose, Cardello and Maller (1981) indicated that satisfactory color in samples of cooked, smoked ham can significantly increase the acceptability of the product even when other quality attributes such as flavor are found to be inferior. Results also indicated that consumer acceptance of ham that was processed in the absence of nitrite was inferior when compared to products cured with added nitrite (DuBose and others 1981). Sebranek, Schroder, Rust and Topel (1977) further emphasized that variation in added nitrite (0, 25, 52, and 156ppm) causes significant differences on the judgment of acceptable color in frankfurters. These differences in the color of frankfurters had an impact on the overall product acceptability, indicating that the samples containing the maximum allowable level of nitrite (156ppm) were significantly more acceptable than those containing 52ppm, and frankfurters containing 52ppm were deemed more acceptable than those produced with 25ppm or no added nitrite. Even so, Wasserman and Talley (1972) found that consumers gave equal preference ratings to cooked nitrite-cured and nitrite-free bacon. It has also been reported (Wierbicki and others 1973, 1976) that characteristic color and flavor can be produced in smoked ham with minimal amounts of nitrite (25ppm), as samples from this study were not found to be significantly different in overall acceptance of smoked ham produced with higher levels of nitrite. Distinguishable differences among products produced with reduced nitrite levels reveal that discrepancies in overall consumer acceptance may be product dependent and that while color does not predict other

quality attributes in certain product categories, the consumer continues to make that association.

Cured Meat Chemistry

Based upon the aforementioned discussions on fresh meat color, it should be of no surprise that the structural adaptability of myoglobin to bind molecules at the sixth ligand plays a crucial role incurred meat color development. Cured meat color is accomplished by supplying nitric oxide (NO) for binding at the sixth position of the heme protein. Providing nitric oxide for cured color development is accomplished by the direct addition of nitrite through various methods depending on the type of product being produced. Nitrite is typically added directly during the chopping, grinding, or mixing process for emulsion type (frankfurters and bologna) and comminuted sausage (dry and semi-dry sausages) products. Addition of nitrite for whole muscle products (ham and bacon) varies in application technique and includes: submersion of muscles in a nitrite containing brine or pickle, injection of muscles with a nitrite containing brine, or direct addition of nitrite on the product surface in the production of dry cured products.

While it is clear that the addition of nitrite in the production process is required for cured meat color, the course of action by which color develops is complex and not fully understood. It is now known that nitrite does not single-handedly act as the nitrosating species or reactive compound to result in cured color. The desirable cured meat color develops from derivatives of nitrite that are produced upon addition of nitrite into the meat system (Pegg and Shahidi 2000). It is believed that the

principal reactive species in meat is the anhydride of nitrous acid, dinitrogen trioxide (N_2O_3), which can react with inherent reductants in the muscle tissue to produce nitric oxide (NO) (Pegg and Shahidi 2000). Due to the fact that nitrite is extremely reactive and the meat itself is a very complex raw material, the curing process includes a multitude of variables which obscure the reactions that take place during the curing process.

The meat system itself undergoes many biochemical changes during the conversion of muscle to meat and one of the most important biochemical changes postmortem with respect to curing is the production of lactic acid in the muscle resulting in a pH decline and development of mildly acid conditions (Sebranek 1980). These acidic conditions play an important role in the reactions that take place when nitrite enters into the meat system. Added nitrite dissolves in the water phase of a meat allowing the nitrite ions (NO_2^-) to react with the slightly acidic conditions of the meat mixture (pH 5.5-6.0). Given that nitrite is a conjugate base, it can gain a hydrogen ion from the meat system to form nitrous acid (HNO_2). The concentration of nitrous acid is very low (0.1-1.0% of added nitrite) in a meat system as the pKa of HNO_2 (3.36) is below the pH of the meat.

Production of nitric oxide from nitrite is a necessary step for cured meat color as it is nitric oxide that reacts with myoglobin to produce cured meat color. The formation of nitric oxide can be influenced by a number of factors, mainly pH and the presence of reductants either naturally present or added as non-meat ingredients in the curing process. A lower pH will increase the formation of nitric oxide from nitrous

acid. Several studies have shown that a small decrease in the pH of the meat system (0.2-0.3 pH units) can double the rate of nitric oxide production and greatly reduced the amount of residual nitrite in the finished product (Fox 1967; Fox 1974; Sebranek 1979). On the other hand, decreases in the production of nitric oxide have also been observed in products with added alkaline phosphates, which increase the pH of the meat system. The pH effect of added phosphates in a meat system are beneficial with regards to water retention properties, however, the higher residual nitrite levels in oven roasted turkey (Ahn and Maurer 1989) and poultry frankfurters (Prusa and Kregel 1985) illustrate the negative effects that an increase pH can have on nitrite conversion and the eventual production of nitric oxide.

The addition of reductants/curing accelerators as non-meat ingredients, commonly in the form of ascorbate and erythorbate, have been found to have a significant impact on the production of nitric oxide and cured color development in meat systems. These curing accelerators possess the ability to provide a reducing environment, which allows for more favorable conditions for the reduction of nitrite, conversion of nitrous acid to nitric oxide, and subsequent reactions of nitric oxide with reduced myoglobin within the meat system (Pegg and Shahidi 2000). The addition of salt has also been found to accelerate the reduction of nitrite to nitric oxide. The chloride ion has the ability to react with nitrous acid and result in the production of the intermediate, nitrosyl chloride. Nitrosyl chloride has been found to be a more reactive nitrosating species than even dinitrogen trioxide (Møller and Skibsted 2002). Sebranek and Fox (1985) showed a linear relationship between

increasing the chloride ion concentration and nitric oxide formation. These results indicate that non-meat ingredients, namely sodium chloride and various curing accelerators have a synergistic effect with nitrite in regard to nitric production and the development of cured meat color.

Cured Meat Color Development

Nitrite acts as a strong heme oxidant, and when added to a comminuted meat system in the presence of oxygen, a browning effect occurs (Pegg and Shahidi 2000). Consequently, when nitrite is added to meat myoglobin and oxymyoglobin are oxidized to metmyoglobin and in turn the nitrite is converted to nitric oxide. When myoglobin in the ferric state it combines with nitric oxide, an intermediate, unstable pigment, nitrosylmetmyoglobin is formed. Nitrosylmetmyoglobin auto-reduces within time, through reducing compounds either naturally present or added to the meat system in the form of non-meat ingredients. This auto-reduction process forms the relatively stable nitrosylmyoglobin or nitric oxide myoglobin which is bright-red in color. The widely accepted and appreciated characteristic “pink” color of cured meats is not formed until the meat product is heated. Upon heating to 130° to 140°F, proteins begin to coagulate. The globin portion of the protein denatures and detaches from the iron atom resulting in the formation of the stable cured meat pigment, nitrosohemochrome. Formation of nitrosohemochrome has been shown to develop at lower temperatures if sufficient time is available (Rust and Olson 1973). A minimum of 2 hours is considered necessary to convert 90% of nitrite to nitric

oxide for later binding on myoglobin and for nitrosylmyoglobin formation (Lee and Cassens 1976) and in turn nitrosohemochrome formation.

In general, nitrosohemochrome is considered a stable pigment, nonetheless under certain conditions changes in the pigment may emerge. Discoloration can result from chemical, physical and microbiological sources. Color degradation from bacterial contamination and growth typically involves off undesirable formation of brown and green pigments. These colors are a result of hydrogen peroxide production and buildup from catalase positive bacteria (Price and Schweigert 1987). The greening phenomenon has been found to be more prevalent in fermented products. The lowered pH provides favorable conditions for peroxides to form pigments that cause detrimental changes in cured meat color (Fox 1966a).

Sources of chemical contamination, more specifically undercure or overcure, may also produce defects in cured color. These discolorations are often referred to as “greening” and in the case of undercure are attributed to the depletion of inherent reductions in the meat system that aid in the conversion of nitrite to nitric oxide. The incidence of undercuring in meat products has been essentially eliminated since the adoption of curing accelerators, namely ascorbate and erythorbate, as non-meat ingredients in the production of cured products. At nitrite levels of 300ppm or more overcuring may occur (Price and Schweigert 1987). Studies conducted by Fox and Thompson (1963) explained that the high levels of nitrite associated with overcure may allow the heme pigment to further react to form a porphrin ring compound called nitrimetmyoglobin. Nitrimetmyoglobin is irreversible and will continue to degrade the

meat pigment upon continued exposure to nitrites, which may result in bright green, yellow or colorless product (Fox 1966a). The occurrence of color changes in meat products due to overcuring is very minimal as a result of the strict regulations set in place for usage of nitrate and nitrite in the production of cured meat products.

With the exclusion of biological and biochemical factors such as bacterial growth and enzymes, the key factors known to cause discoloration are oxygen, light and dehydration (Draudt and Deatherage 1956). Long exposure to high intensity light or oxygen will cause the cured meat pigment to develop a tan, grayish-brown appearance (Rust and Olson 1973). Color fading, is thought to occur in a two step sequence. First, light accelerates the dissociation of nitric oxide from the myoglobin pigment and is catalyzed in the presence of oxygen. Dissociation and oxidation of nitric oxide soon follows (Tarladgis 1962; Fox 1966a). As a result, oxygen penetration or presence becomes a significant factor in discoloration of cured meat products, especially if light is present. Discoloration in light-displayed packaged products starts near the edge of the product surface where there is less package adhesion and greater residual air space (Lin and Sebranek 1979). It is critical to optimize storage conditions in cured meat products to prevent color degradation. Packaging cured meat products with exposed cut surfaces using vacuum packaging systems with films that possess low oxygen permeability are keys to maintaining acceptable cured color.

Flavor Development and Protection

Nitrite is unique in its ability to provide not only desirable color characteristics for the consumer, but also a pleasing flavor profile as well. The responsibility of nitrite in the development of cured meat flavor is difficult to decipher, and the chemical changes that are responsible for the distinctive flavor are not clearly understood (Shahidi 1998). The first observation of a relationship between nitrite and cured meat flavor was established by Brooks in 1940 when it was found that as little as 10ppm of nitrite in combination with salt alone could develop a significant cured meat flavor in bacon and hams. This disagrees with later research that indicated that a minimum of 50ppm of nitrite was required to develop significant cured meat flavor (MacDonald and others 1980).

Conflicting results on the impact of added nitrite on cured meat flavor have continued to occur. Studies conducted by Huhtanen and others (1980) and Wasserman and others (1977) found no differences in untrained panelist's preference between nitrite-free and nitrite-cured bacon. These results may be attributed to the effects of sodium chloride on cured meat products. Kimoto and others (1976) indicated that sodium chloride was more important to the flavor of bacon than was nitrite. These results support those seen by Greene and Price (1975), who found that ground samples containing nitrite alone produced very little cured meat flavor.

The synergistic effects of salt and nitrite on flavor may be product dependent. In a study conducted by Cho and Bratzler (1970), significant differences were

detected in cured pork loin samples. Blindfolded panelists could distinguish that pork with sodium nitrite had more “cured flavor” than those that did not contain nitrite in the formulation. Frankfurters produced with varying levels of nitrite (0, 78 and 156ppm) showed similar results in sensory evaluations. Panelists were able to detect flavor differences between frankfurters produced with no nitrite and 78ppm of nitrite. Panelists also indicated a trend between flavor of frankfurters produced with 78ppm and 156ppm nitrite (Wasserman and Talley 1972). Subsequent studies showed that sensory panelists found significant flavor differences in frankfurters with varying levels of nitrite (0, 39, 78 and 156ppm) (Simon and others 1973). Frankfurters with increased levels of nitrite tended to be different, but the lack of significance between 39 and 78ppm nitrite can be attributed to the addition of curing accelerators in the formulation. Curing accelerators have been found to increase the overall flavor and acceptability of frankfurters with low (below 52ppm) concentrations of nitrite (Sebranek and others 1977).

While the exact mechanism behind nitrite’s impact on flavor is unknown, Shahidi (1998) indicated that the inhibitory effects that nitrite has on lipid oxidation aids in the development of cured flavor. Deterioration or oxidation of lipids is generally accepted as being the primary process by which loss in quality of meat and meat products occurs. Warmed over flavor (WOF), a term first describe by Tims and Watts (1958), is used to illustrate the off-flavor that develops rapidly in refrigerated uncured cooked meats through oxidation of unsaturated fatty acids. Lipid oxidation of unsaturated fatty acids includes initiation, propagation and

termination phases and occurs in the presence of oxygen. Initiation of lipid oxidation occurs when a hydrogen atom (H) is eliminated from an unsaturated fatty acid (RH) by bonding with oxygen (O_2) or other catalysts to form a lipid free radical ($R\bullet$). Shortly thereafter, the propagation step begins where the formation a peroxy radical ($ROO\bullet$) results from the reaction of a lipid free radical ($R\bullet$) with oxygen. The peroxy radical triggers a cascading effect upon formation as these peroxy radicals have the ability to eliminate a hydrogen atom from another unsaturated fatty acid molecule producing a hydroperoxide ($ROOH$) and a new lipid free radical ($R\bullet$). Free radicals return to the propagation step, and react with oxygen; triggering an incessant cycle of hydroperoxides and free radical formation until either oxygen or unoxidized lipids are depleted (Pegg and Shahidi 2000). Autooxidation of unsaturated fatty acids can occur in uncured cooked meats within 48 hours at 4°C (Price and Schweigert 1987) however, this phenomenon does not occur in cured meats due to the antioxidant effect of nitrite (Fooladi and others 1979; MacDonald and others 1980). It is not clear whether nitrite or nitric oxide is the actual antioxidant in cured meat or if it is a by-product of the various reactions that take place during the curing process (Pegg and Shahidi 2000). Pearson, Love, and Shorland (1977) hypothesized that nitrite may either stabilize the lipid components or inhibit the prooxidants normally present in muscle tissue. Igene, Yamauchi, Pearson, and Gray described three possible methods by which nitrite acts as an antioxidant (1985):

- (1) Formation of a strong complex with heme pigments, thus preventing the release of non-heme iron and its consequent means of lipid oxidation;
- (2) Direct interaction with the reduced non-heme iron (Fe^{2+}) from denatured heme pigments;
- (3) Through stabilization of the unsaturated lipids within the membranes.

While these researchers felt that preventing the release of Fe^{2+} during the cooking was critical, more recent research emphasized the stabilization of unsaturated fatty acids to explain the antioxidant role of nitrite in cured meats. Phospholipids from cured pork were found to react with nitrite or dinitrogen trioxide to form nitro-nitroso derivatives, which in turn were capable of stabilizing lipids susceptible to oxidation (Freybler and others 1993).

Antimicrobial Properties

The beneficial uses of nitrite thus far have focused on the initial reason that it was used in the manufacture of cured meats; to produce an appealing color and flavor in the finished product. While the qualitative benefits of color and flavor of cured meats are quite noticeable for consumers to recognize and appreciate, the ability of nitrite to inhibit the outgrowth of certain bacteria is far more important and valuable. Nitrite is a bacteriostatic agent that slows the growth of both spoilage and pathogenic bacteria. Nitrite is a strong inhibitor of anaerobic bacteria and has been

shown to contribute to the control of other pathogens such as *Listeria monocytogenes* (Tompkin 1995). Nitrite affects different microorganisms in diverse ways and the mechanisms for inhibition vary between microorganisms. While nitrite contributes to the control of pathogens including *Listeria monocytogenes*, it is most noted for possessing the ability to inhibit the growth of spore forming pathogens, particularly *Clostridium botulinum*.

The earliest reference to *C. botulinum* can be traced back to Emperor Leo VI in 886-912 AD, when he banned the manufacture and consumption of blood sausage due to its harmful health effects (Jay and others 2005). This suggestion of botulism was termed “sausage poisoning” prior to the identification of the causative agent until 1896 when a scientist isolated the organism from raw, salted, unsmoked ham that was a vehicle of a recent outbreak. Van Ermegen named the organism *Bacillus botulinus* after the Latin word for sausage, *botulinus* (Jay and others 2005). While the incidence of human botulism has declined in modern times, *Clostridium botulinum* remains one of the most important and deadly pathogenic foodborne bacteria.

Clostridium botulinum is a gram positive, anaerobic, sporeforming, rod-shaped bacteria that is indigenous to soils and waters (Jay and others 2005). Strains of *C. botulinum* produce characteristic botulinal neurotoxins (BoNT) that cause the condition known as botulism. There are seven distinct toxigenic forms of BoNT, designated types A, B, C, D, E, F, and G. Outbreaks of foodborne botulism are typically caused by types A, B, E, and in rare instances type F (Boerema and

others 2004). The botulinal neurotoxins are the most toxic substances known to man, and it is estimated that as little as 0.1-1 μ g of BoNT is lethal to humans (Boerema and others 2004; Jay and others 2005). Upon ingestion, the toxin is absorbed through the walls of the stomach and intestines and enters the bloodstream. BoNT then penetrates the nervous system where it blocks the release of acetylcholine, the neurotransmitter responsible for muscle contraction (Boerema and others 2004). The blocking of acetylcholine at nerve-muscle junctions leads to flaccid paralysis, which has led to alternative uses for BoNT for cosmetic purposes (Jay and others 2005). When injected intramuscularly at therapeutic doses, BoNT type A is now commonly recognized as an integral component of facial rejuvenation treatments that reduce frown lines by localized reduction of muscle activity (Carruthers and Carruthers 2005).

While botulinal toxins may show great promise for both cosmetic and therapeutic medical purposes, the repercussions of toxin formation in meat and poultry products is far more severe. Foodborne botulism can only occur upon consumption of foods in which *Clostridium botulinum* has grown and produced the toxin. Symptoms of botulism occur between 12 and 72 hours after ingestion of the toxin containing food and consist of nausea, vomiting, impaired vision due to paralysis of the ocular muscles, difficulty speaking and swallowing, gastrointestinal disturbances, muscle weakness and respiratory failure. Fatal causes are typically the result of respiratory failure and the mortality rate ranges between 30 and 65%. Deaths from botulism have fallen in recent years due to improved medical care and

timely administration of the antitoxin (Boerema and others 2004; Carruthers and Carruthers 2005; Jay and others 2005).

Vacuum packaged processed meat products serve as a suitable medium for the outgrowth of *C. botulinum*, as this pathogen thrives in conditions without oxygen. Growth begins between a pH range of 4.6 and 8.8 and a temperature range of 3.3°C to 55°C with an optimum growth temperature near 37°C (Jay and others 2005). Nonetheless, there have been reports of botulinal toxin growth as low as 2.9°C in culture media and 2.0°C in vacuum packs (Boerema and others 2004).

While *C. botulinum* is a strict anaerobe, the redox potential of the product itself is often low enough to support growth of the organism and toxin production even when oxygen is present (Fernandez and others 2001). Modified atmosphere packaging is often used to flush remaining oxygen out of the vacuum package with gases such as CO₂ to remove residual oxygen and extend shelf life. Investigations of modified atmosphere packaging on *C. botulinum* have yielded mixed results. In a study conducted by Fernandez and others (2001), a concentration of 5% CO₂ enhanced growth, 50% CO₂ exhibited moderate inhibition and a concentration of 90% CO₂ had a considerable inhibitory effect on the growth of spores. While Lövenklev and others (2004) agreed that a high concentration (70%) of CO₂ decreased the growth rate, they also found that neurotoxin expression and BoNT production greatly increased. Consequently, modified atmosphere packaging may result in an increased risk for botulism through stimulation of toxin production.

Nitrite is particularly effective against *Clostridium botulinum*, and its use in processed meat production has virtually eliminated the risk of this deadly pathogen.

As with the case of color and flavor development, the antibotulinal mechanism is not clear, but microbial inhibition is thought to be a result of nitrous acid derived from nitrite in the acid environment of meat systems. The effectiveness of nitrite against *C. botulinum* is dependent upon several factors including spore level, ingoing nitrite level, pH, concentration of sodium chloride, iron content, presence of reducing agents and residual nitrite levels in the meat (Tompkin 1995; Archer 2002). Pierson and Smooth (1982) indicated that the antibotulinal effects of nitrite occur at two different stages, the first in controlling the inhibition of vegetative cells emerging from surviving spores and the second is by preventing cell division and growth in any vegetative cells that do emerge from surviving spores. The reactions of nitric oxide appear to be responsible for this effect, further emphasizing the importance of nitrite chemistry. Vegetative cells of *C. botulinum* were found to contain iron-sulfur proteins (Reddy and others 1983) and the reactions of nitric oxide have been shown to reduce germination by inactivating iron-sulfur complexes that are essential for growth (Payne 1990).

IV. Residual Nitrite

Nitrite is extremely reactive upon addition to a meat system and can react with proteins, lipids, meat pigments and other components of meat (Pegg and Shahidi 2000). Chemical reactions and binding of nitrite to meat proteins is exacerbated when heat from thermal processing procedures is applied. Nevertheless, a portion of the added nitrite remains unreacted and unbound and is referred to as residual nitrite, which can be measured analytically (Pegg and Shahidi

2000). Depletion of nitrite initiates instantaneously upon the addition to meat and is continuous throughout storage (Pearson and Gillett 1996). Post processing, only 10-20% of the ingoing nitrite can be analytically measured (Cassens 1997b). These residual levels continue to deplete throughout storage until undetectable levels are present in the finished cured meat product (Pearson and Gillett 1996).

The concentration of residual nitrite is dependent upon a multitude of factors including pH, presence of reductants, muscle type and temperature of the meat system upon addition (Pegg and Shahidi 2000). The presence or addition of curing accelerators including ascorbate and erythorbate, serve as reducing agents which in turn accelerate the curing reaction resulting in lower levels of residual nitrite (Lee and Shimakoa 1984). Lee, Cassens and Fennema (1976) demonstrated that lower pH levels in the meat system resulted in lower levels of residual nitrite. Research conducted by Sebranek was consistent with these findings and further explained that the reactivity of nitrite increases with decreasing pH, resulting in lower concentrations of residual nitrite. In fact, a small decrease in pH, as low as 0.2 pH units can double the rate of color formation in more favorable interactions with myoglobin (Sebranek 1979). Addition of non-meat ingredients including alkaline phosphates and the addition of poultry meat in the formulation has also been found to alter overall product pH in turn effecting residual nitrite levels (Prusa and Kregel 1985).

The question still remains whether ingoing nitrite or residual nitrite is more important for food safety. It has been established that products with higher levels of

ingoing nitrite are less likely to result in the outgrowth of *C. botulinum* (Christiansen and others 1973). Addition of sodium ascorbate increases the intensity of the reaction, by generating more nitric oxide, and thereby increasing the antibotulinal efficacy of nitrite in cured meat products (Reddy and others 1983).

V. Nitrite and Human Health

Toxicity

Despite its proven track record for food safety, nitrite usage in the production of meat products has been controversial for decades. Nitrate and nitrite are compounds that can be toxic to humans when not used appropriately. Levels above 300 mg/kg of body weight is considered the lethal dose for nitrite and is reason for the strict regulations in place to control and monitor nitrite usage in meat products. Levels used in cured meat production and consumption of these products do not present a known health hazard (Pierson and Smooth 1982). Nevertheless, if nitrite is consumed by itself it can cause serious health implications or even death through the condition known as methemoglobinemia. Characteristics of methemoglobinemia include the distinguishing blue color of the skin that is a direct result of high amounts of unoxygenated hemoglobin present, indicating a lack of oxygen in the blood as well as other organs and tissues in the body. Hemoglobin is unable to transport and release oxygen to organs and various tissues in the body as a result of becoming oxidized from the ferrous to the ferric state. Newborn infants are particularly susceptible to methemoglobinemia as the enzymes needed to counteract the effects of nitrite poisoning are not fully developed. This leads to “blue baby syndrome” in

which the infants turn blue in color due to lack of oxygen to internal organs and, if severe enough, can be fatal (Cammack and others 1999b). For this reason, the USDA does not allow nitrates or nitrites in baby, junior, or toddler foods (USDA 1995). While cases of nitrite induced methemoglobinemia are typically caused by accident, reports of suicide (Hill 1991) and intentional poisoning have occurred. To prevent the addition of curing agents improperly and possible nitrite poisoning, the meat industry has adopted the use of curing mixes, in which low levels of sodium nitrite are blended with salt and red dye to result in a pink color, clearly distinguishing it from salt, sugar, or other non-meat ingredients.

Nitrosamines and Cancer

Repeated concerns have arisen in both popular and scientific literature regarding the extent and effects of nitrate and nitrite in the human diet. A vast number of concerns with nitrite usage in meat products stem from reports that link the consumption of nitrite containing foods, particularly processed meats, to various types of cancer. Problems first arose in the 1970's when it became evident that nitrite-cured meats, more specifically bacon, had the potential to produce nitrosamine compounds, which have been found to be carcinogenic (Cassens 1995).

Nitrite is extremely reactive and under appropriate conditions can serve as a nitrosating agent to produce nitrosamine compounds. It is not the nitrite itself, but rather one of its derived by-products, nitrous acid, which has the ability to react with secondary amines to result in the formation of nitrosamines (Wolff and Wasserman 1972; Pegg and Shahidi 2000). These dangerous nitrosamine compounds can be

formed when foods containing nitrite are exposed to high temperatures (Ahn and others 2002) and may also be formed when nitrates react with amines in gastric acids in the stomach (Archer 2002). Many of these carcinogenic compounds have been suggested to induce tumors in the human body if present at appropriate concentrations (Ahn and others 2002). Nevertheless, no link has been established between nitrate or nitrite consumption and cancer to date. In fact, in a study conducted by Maekawa and others (1982), which continuously administered sodium nitrate and nitrite to rats over a two year period, concluded that no carcinogenic activity or nitrosamine generation occurred as a result of high intake levels of these ingredients. Eicholzer and others (1998) explored the link between brain, esophageal and nasopharyngeal cancers to dietary nitrates, nitrites and nitroso compounds and found no epidemiological evidence to make a conclusive link of intake to cancer. When found in cooked cured meats nitrosamines are present in very small quantities, typically in the parts per billion (ppb) range, if detected at all. Nonetheless, due to a potential link of these compounds to various types of cancer in humans, the presence of nitrosamines in cured meat products have received a great deal of attention over the years.

Bacon is of particular concern as the combination of cooking at high temperatures and the presence of secondary amines and residual nitrite has made it difficult to eliminate the issue completely. Lowering ingoing nitrite levels, addition of curing accelerators as well as reducing residual nitrite have been three methods that have received special attention. In fact, concerns with nitrosamine formation

resulted in USDA regulations prohibiting the use of nitrate in bacon products. Additionally, maximum allowable levels are reduced to 120ppm and must be supplemented with the maximum level of curing accelerators including ascorbate and erythorbate, to reduce the amount of residual nitrite in the finished product (USDA 1995). Ascorbate and erythorbate reduce nitrous acid, therefore pushing the reaction away from the formation of nitrosamines (Townsend and Olson 1987).

Fears during the 1970's became so great, that the U.S. government even considered a total ban on nitrite usage in meat products (Pegg and Shahidi 2000). Nitrite was never banned, as it is a dynamic compound that provides desirable color and flavor, but also public protection from deadly foodborne pathogens. To date, an equivalent substitute for nitrite has not been found. From the time since the nitrosamine issue first arose, the meat industry has made significant changes to prevent and essentially eliminate the risk of nitrosamine formation in cured meat products. With the exception of specialty products the use of nitrate has been eliminated, usage levels of nitrite have decreased and maximum levels of ascorbate and erythorbate were used to inhibit the formation of nitrosamines (Cassens 1997a).

Even so, the relationship between cured meat consumption and cancer is still making headlines today. Peters and others reported (1994) that children who ate more than 12 nitrite-cured hot dogs per month showed an increased risk for developing childhood leukemia. Most recently, the 2007 World Cancer Research Fund (Cancer Project) released a report citing controversial science and scare tactics to alarm consumers about processed meats, especially hot dogs. As part of

their vegan agenda, a petition was submitted to the U.S. Department of Agriculture to change the Child Nutrition Act and remove hot dogs and other processed meats from the National School Lunch Program ([AMI] American Meat Institute 2008). The Cancer Project chose to ignore prominent studies that showed no cancer link to hot dogs and processed meats. In fact, the report completely omitted one of the largest studies performed involving over 725,000 men and women that showed that greater intake of either red meat or processed meat was not related to colorectal cancer risk (Cho and others 2004). Regardless of the debates that will continue to occur over the role of nitrite-cured meat products to various types of cancer, it is important to reiterate the fact that no known case of human cancer has ever been shown to result from exposure to nitroso compounds (Pegg and Shahidi 2000).

Exposure and Sources of Dietary Nitrate and Nitrite

While nitrite is an integral ingredient in the production of cured meat products, it is not the sole or primary source of nitrite in the diet. In fact, nitrite is most commonly ingested upon the consumption of vegetables (Archer 2002). Nitrates and nitrites are part of the nitrogen cycle of plants and are by-products of photosynthesis (Bednar and Kies 1994). Nitrate is necessary for growth (Hill 1991), and nitrogen based fertilizers, genetics of plants and the environmental conditions can play a role in the amount of nitrate found in foods (Wolff and Wasserman 1972). Concentrations of nitrates in vegetables are extremely variable, ranging from around 1 to 10000mg/kg fresh weight (Hill 1991). The National Academy of Sciences stated that vegetables account for 85% of dietary nitrate (National Academy of Sciences,

1981). Nitrate levels of beets, celery and lettuce have been reported at levels of 2600ppm, 1500ppm and 1700ppm respectively (White 1975). Thus, a person is much more likely to consume as much, but quite often more nitrates from vegetables than from cured meats (Richardson 1907).

Nitrite can be formed *in vivo* through the reduction of nitrate by the combination of certain species of bacteria present in the oral cavity and the acidic properties of saliva. Such nitrate-derived nitrite can be the main source of exposure to nitrite and can easily account for over 90% of ingested nitrite (Archer 2002). This is especially true since nitrite ingestion in humans is ordinarily low when compared to nitrate ingestion. The National Academy of Sciences (1981) reported that 39% of dietary nitrite was from cured meat, however this has since been disputed by the American Meat Institute Foundation scientists that reported less than 5% of nitrite comes from cured meats ([AMI] American Meat Insititute 2003). Therefore, it is likely that the majority of internal nitrite is derived from ingested nitrate via vegetable sources (Hill 1991).

It should also be mentioned that dietary nitrite has also been promoted as beneficial to human health. Similar to the role in cured meat color development, nitrite serves as a reservoir for nitric oxide which is an important physiological messenger in the human body which helps to regulate blood pressure, immune response, wound repair, and neurological functions (Archer 2002).

VI. Uncured, No-Nitrate-or-Nitrite-Added Meat Products

Rationale

The clean eating craze of the last decade has had a significant impact of the availability of natural and organic processed meat products to appeal to health conscious consumers. Uncured, no-nitrate-or-nitrite-added processed meats have been a very significant part of the explosive market growth that is occurring in natural and organic foods (Sebranek and Bacus 2007). It has been estimated that since 1990, organic food sales have increased nearly 20% each year. While meat, poultry and seafood is the smallest sector of the natural and organic foods market, comprising only 2.5%, it is the fastest growing category (Winter and Davis 2006). Even with the current recession, the popularity and stability of organically produced foods on the market remains. In fact, a 2009 survey indicated that only 3 percent of consumers have stopped buying organic products all together and 40 percent of organic consumers haven't changed their purchasing habits during the economic downfall (Mintel 2009).

Organic and natural consumers have very strong opinions on feed production practices, animal husbandry methods, as well as ingredient usage in organic and natural meat products. The negative perception of nitrite as a preservative in conventionally processed meat products held by such consumers may have in part influenced the wide-spread acceptance of "uncured" natural and organic versions of typical cured meats (Sebranek and Bacus 2007). While the "less is more" and "preservative free" consumer approach with respect to ingredient usage has allowed

for a better perception of processed meat products, there is no evidence to support that natural and organic meats are in fact “healthier” and “safer” than conventionally processed meat products. An in depth review conducted by Dangour and others (2009) found that there were no significant nutritional differences between organic and conventionally produced foodstuffs.

Nevertheless, the enhanced demand for minimally processed products has driven meat processors to eliminate or find alternative sources for non-meat ingredients as natural and organic foods must be produced according to the stringent USDA regulations that define these unique product categories. In the case of processed meat products such as frankfurters, ham and bacon, which are typically cured by the addition of nitrate or nitrite, significant changes must occur in the production process as the direct addition of these formulated preservatives are not permitted.

Definitions of Natural, Organic, and Uncured Meat and Poultry Products

According to the Code of Federal Regulations Title 9, Part 317.17 and 319.2, meat products to which nitrate or nitrite is permitted or required can also be manufactured without nitrates or nitrites and must be labeled accordingly. The Code of Federal Regulations (Code of Federal Regulations 2007a, 2007b) states the following:

“Any product which is required to be labeled by a common or usual name for which there is a standard and to which nitrate or nitrite is

permitted or required to be added may be prepared without nitrate or nitrite and labeled with such common or usual name or descriptive name when immediately preceded with the term 'Uncured' as part of the product, provided that the product is found by the Administrator to be similar in size, flavor, consistency and general appearance to such product as commonly prepared with nitrate or nitrite, or both.

Products which contain no nitrate or nitrite shall bear the statement 'No Nitrate or Nitrite Added.'

Products described shall also bear the statement 'Not Preserved-Keep Refrigerated Below 40°F At All Times' unless they have been thermally processed to F_0 3 or more; they have been fermented or pickled to pH of 4.6 or less; or they have been dried to a water activity of 0.92 or less.

Products shall not be subject to the above mentioned labeling requirements if they contain an amount of salt sufficient to achieve a brine concentration of 10 percent or more."

The aforementioned definitions were developed as a response to the multitude of concerns that arose in the 1970's regarding the formation of nitrosamines in meat products. Regulatory agencies felt that if manufacturers had the desire to produce products that were typically cured without the addition of nitrates or nitrites, such products could be produced, bearing the appropriate labeling claims. While this is

the true intention of the labeling guidelines, “uncured” processed meats currently available to consumers possess characteristics similar to conventionally processed products (Sebranek and Bacus 2007). Studies conducted by Sindelar (2006) confirmed that a large majority of commercially available natural and organic processed meats demonstrate typical cured meat properties including color and flavor, which can only be produced by the addition of nitrate or nitrite. In addition to the aforementioned characteristics that appeal to the consumer, chemical analysis of commercially available uncured no-nitrate-or-nitrite-added hams, bacon and frankfurters revealed significant concentrations of residual nitrite and nitrate. Residual nitrate levels ranged from 6.8 to 44.4ppm and residual nitrite levels ranged from 0.9 to 9.2ppm (Sindelar 2006). These results give a clear indication that nitrate or nitrite is being introduced indirectly as components of other ingredients used in the production process.

In April of 2006, the FSIS Labeling Division announced verbal changes to the labeling policies regarding uncured meat products. These changes were as a result of a rapid increase in the growth and sales of this unique product category, realization that nitrates and nitrites may in fact be present, and that the labels may not be truthful and misleading to consumers. In turn, all new labeling submissions must include the disclaimer:

“No nitrates or nitrites added except those found naturally occurring in...”

Celery juice powder, beet juice powder, carrot juice concentrate, sea salt, or any ingredient determined to contain nitrates or nitrites by USDA.

To further clarify the issue, the “No Preservatives” statement could no longer be used. Even with the new policies, the term “uncured” has been questioned by many as to whether it is an appropriate term for this unique class of meat products. Sebranek and Bacus (2007) suggested that the terminology “alternatively cured” or “naturally cured with” may be a more suitable nomenclature for these products that are manufactured with the intention of replacing nitrite.

Additional Processing Considerations

It is obvious that the current regulations for this unique category of products may be inaccurate and misleading to consumers. The term uncured is often paired with other disclaimers, such as natural and organic, further adding to the confusion (Sindelar 2006). If products are labeled natural or organic a separate set of processing procedures and ingredient limitations apply. By definition neither natural nor organic products can contain the direct addition of nitrite. Thus, all organic and natural products are uncured, but not all uncured products are natural or organic (Bacus 2006).

Organic Processed Meat Products

Organic products are well defined and strictly regulated by a set of policies that are carried out by the USDA Organic Foods Production Act (OFPA) (Winter and Davis 2006). Labeling qualifications for organic products are based upon the percentage of organic ingredients in a product. All ingredients are clearly defined as permitted or prohibited in the OFPA National List. Organic products can be labeled

as “100% organic” and products must thereby contain only organically produced ingredients and processing aids. Products which bear the term “organic” must consist of at least 95% organically products ingredients, and all remaining ingredients must be approved on the National List. Products meeting the requirements for “100 percent organic” and “organic” may display these terms and the percentage of organic content on their principal display panel in addition to the USDA Organic seal. Organic products may also display the statement “made with organic ingredients” if the product contains at least 70% organic ingredients, however, the USDA Organic seal cannot be used anywhere on the package. If products contain less than 70% organic ingredients they cannot use the USDA seal or term organic anywhere on the principal display panel. However, they may identify the specific ingredients that are organically produced in the ingredients statement. In addition to strict guidelines for labeling and allowable ingredients, the National Organic Program developed and implemented standards in 2002 that further specified the methods, practices and substances that may be used for production, processing and handling of animals used for the production of organic meat products (Winter and Davis 2006). As a result, all raw materials must come from animals that are raised under organic management at a USDA-certified farm in addition to all ingredients being approved for use by the OFPA National List.

Natural Processed Meat Products

The use of the term “natural” on meat products has not been as clearly defined by the USDA which allowed for more flexibility and controversy within the

product category. The first form of guidance for “natural” labeling claims was outlined in Standards and Labeling Policy Memorandum (Memo) 055, dated November 22, 1982. Policy Memo 055 stated that the term “natural” may be used in the labeling of meat and poultry products provided that the applicant for such labeling demonstrates that:

“(1)The product does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 CFR 101.22), or any other artificial or synthetic ingredient; and

(2)The product and its ingredients are not more than minimally processed. Minimal processing may include: (a) Those traditional processes used to make food edible, to preserve it, or to make it safe for human consumption, e.g., smoking, roasting, freezing, drying, and fermenting, or (b) those physical processes that do not fundamentally alter the raw product or that only separate a whole, intact food into component parts, e.g., grinding meat....

Relatively severe processes, e.g., solvent extraction, acid hydrolysis, and chemical bleaching, would clearly constitute more than minimal processing.”

Policy Memo 055 also indicated that the use of an ingredient that has undergone more than minimal processing would prevent a product in which the ingredient is used from bearing a “natural” claim. This became of particular

importance in August of 2005 when FSIS withdrew Policy 055 and incorporated its policy on “natural” into the Food Standards and Labeling Policy Book (USDA 2005). The original policy was modified to acknowledge that that “sugar, sodium lactate (from a corn source) [at certain levels], and natural flavorings from oleoresins or extractives are acceptable ingredients for ‘all natural’ claims.”

Manufacturing Methods for Uncured, No-Nitrate-or-Nitrite-Added Meat Products

Producing organic and natural processed meats are especially challenging when considering the multitude of ingredients that are not permitted in these products. A study conducted by Sindelar and others (2007a), found that products with the intention of replacing nitrite and provide characteristics that are similar to conventional cured meat products, utilize a manufacturing process in which naturally occurring nitrates are obtained from vegetable sources. Commercially available vegetable powders are also offered to meat processors and can provide nitrate in concentrated form and concentrations of 27,462ppm nitrate have been reported in commercially prepared celery juice powder (Sindelar and others 2007a). An analysis of commercially available vegetable juices reported that carrot, celery, beet, and spinach juice contained 171ppm, 2114ppm, 2273ppm, and 3227ppm of nitrate respectively (Sebranek and Bacus 2007). While it is obvious that vegetables such as beets and spinach would provide a sufficient nitrate source for these minimally processed foods, the resulting sensory properties would be objectionable to consumers. Concentrated celery powders have been successful in providing both a

compatible flavor profile to meats as well as an adequate concentration of nitrate for curing to take place.

The addition of a nitrate containing source is only a portion of the “natural”, “alternative” or indirect curing equation. Nitrate must first be reduced or converted to nitrite before being readily available to engage in subsequent steps of the curing reaction. Commercial grade starter cultures are available which contain nitrate reductase positive microorganisms which allow the added nitrate from the vegetable source to be converted to nitrite. Coagulase negative (non-pathogenic), catalase positive staphylococci, most commonly *Staphylococcus carnosus*, is most universally used. It is well recognized that most species of staphylococci possess enzymes involved in nitrate/nitrite metabolism (Neubauer & Götz, 1996). Talon and others (1999) measured the nitrate reductase of several different strains of staphylococci isolated from dry sausage. Authors found that strains of *Staphylococcus carnosus* had the highest nitrate reductase activity of all staphylococci tested. These cultures were typically used for old world dry sausage products in which nitrate is used to produce nitrite throughout the long curing process, drying, and storage periods (Bacus 1984; Sebranek and Bacus 2007).

Production of dry and semi-dry meat products allowed for sufficient time for nitrate to nitrite conversion during the production process, however, today's high speed processing facilities and typical manufacturing methods of ready-to-eat (RTE) meat products would not allow ample time for the conversion of nitrate to nitrite to take place. When investigating the effects of varying levels of vegetable juice

powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham Sindelar and others (2007b), found that incubation time was more critical than the amount of vegetable powder to produce products which were similar to the nitrite added controls. Interestingly, this incubation period was more critical in small diameter emulsified sausages than in larger diameter uncured, no-nitrate-or-nitrite-added hams. Sindelar concluded that the longer come up times in the thermal processing schedules allowed for sufficient time for the conversion of nitrate to nitrite to take place, where as the incubation period was necessary for small diameter products which would reach their maximum internal temperature at a much faster rate (Sindelar and others 2007b, 2007c).

Ingredient technologies in the production of uncured, no-nitrate-or-nitrite-added meat products have progressed rapidly with the increased demands for these products and a new era of vegetable powders have emerged. Pre-converted celery powders are currently available in which the active ingredient is nitrite and in turn requires no starter culture or additional processing time. In these powders, the *Staphylococcus carnosus* starter culture is added to the celery puree and the conversion takes place in a carefully monitored system. The puree is then dried and the preconverted powders ultimately contain 10,000 – 15,000ppm nitrite and are added at levels between .2 - .4%. Usage levels result in approximately 20-40ppm of ingoing nitrite and while these powders may seem more ideal from a production standpoint, they raise additional concerns in regards to safety.

Concerns with preconverted celery powders are triggered by research conducted by Sen and others (1973). Authors reported occurrence of fairly high levels of nitrosamines in meat curing mixtures containing spices and nitrite. The authors hypothesized that some of the nitrosamines in cured meat products may originate from these curing mixtures as nitrosamines form due to the interaction of amines in spices and nitrite in the premixed spice blends under dry conditions (Sen and others 1973). As a result, nitrite containing spice blends are prohibited and have been discontinued in both the United States and Canada. With the recent release of such preconverted vegetable juice powders, concerns arise as the risk of formation of nitrosamines in these preconverted celery powders have not yet been established.

Additional concerns in regards to safety circle around the toxicity of nitrite and the lack of regulation placed on these preconverted powder systems. Chemically derived nitrate and nitrite are strictly controlled, restricted and monitored by the USDA. Additional considerations may need to be addressed in facilities that are producing no-nitrate-or-nitrate-added meat products to ensure the appropriate levels of preconverted celery powders and nitrate containing powders are being added to meat products.

VII. Concerns Associated with Uncured Meat Products

Food related illnesses, hospitalizations and deaths due to contamination of RTE meat products continue to be a significant problem in the United States. Processors are impacted by economic losses despite the multitude of hurdles and

intervention strategies utilized throughout the production process. Approximately \$152 billion dollars in financial losses are caused by the estimated 76 million foodborne illnesses that occur annually in the United States (Scharff 2010; Mead and others 1999). Of those individuals who become ill, approximately 300,000 are hospitalized and 5,000 die. Five pathogens; *Salmonella*, *Listeria*, *Toxoplasma*, *Norwalk*-like viruses, *Campylobacter* and *E. coli* O157:H7, account for over 90% of estimated food-related deaths. Nearly 28% of these deaths are associated with listeriosis caused by consumption of RTE foods contaminated with *Listeria monocytogenes* (Mead and others 1999). In a risk assessment published in 2003, the U.S. Food and Drug Administration and the U.S. Food Safety and Inspection Service estimated that about 90% of human listeriosis cases in the United States are caused by consumption of contaminated deli meats (Food and Drug Administration and others 2003) making RTE meat products a high risk product for possible contamination with *L. monocytogenes*.

Listeria monocytogenes

There is no doubt that *Listeria monocytogenes*, the pathogen responsible for listeriosis, has emerged as a serious problem in RTE processed meat products and represents a considerable public health concern. Despite the fact that the occurrence of listeriosis is rare, accounting for only 2,518 cases of foodborne illness, *L. monocytogenes* carries one of the highest mortality rates (~30%) of any foodborne pathogen (Mead and others 1999). While consumers often scrutinize the preservatives and non-meat ingredients that are used in many conventionally

processed meat products, they are extremely important when assessing the potential for growth of deadly pathogens including *L. monocytogenes*. It is important to fully understand factors that affect the growth, inhibition, inactivation and survival of *L. monocytogenes*. A better understanding will allow for improvement in safety, reduce the risk for contamination and ensure that food is safe from *L. monocytogenes*.

Background and History

L. monocytogenes was first described by E. Murray in 1926 based on six cases of sudden death in young rabbits. Murray referred to the organism as *Bacterium monocytogenes* after typical monocytosis was observed in the blood from infected rabbits (Murray and others 1926). It was later discovered that the same organism was isolated in South Africa and named *Listerella hepatolytica* in 1927. This led to the merging and change in name to *Listerella monocytogenes*. Eventually in 1940 the name was amended to the presently known *Listeria monocytogenes* (Pirie 1940). Until 1961, *L. monocytogenes* was the only species recognized in the genus *Listeria*, however, there are currently six species recognized which include: *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi* (Buchrieser and Rocourt 2009). *L. monocytogenes* is the primary pathogenic species and currently 13 serotypes can be distinguished; 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 5, 6a, and 6b. Of these, 1/2a, 1/2b, and 4b, have been implicated in over 90% of cases in humans (Gellin and others 1991; Buncic and others 2004).

Presence of *L. monocytogenes* in Foods and in the Environment

L. monocytogenes has been isolated from many different settings including: soil, water, vegetation, sewage, animal feed, farm environments, and food-processing environments (Wiedmann and Saunders 2009) in addition to the intestinal tracts of healthy animals and humans (Jay and others 2005). Because of its ubiquitous nature in the environment and ability to survive adverse conditions for long periods of time in the environment, on foods, in processing plants and household refrigerators there are multiple modes for transmission of *Listeria monocytogenes* to meat products. Although frequently present in raw foods of both plant and animal origin, it also can be present in cooked foods due post-processing contamination.

Factors Affecting the Growth and Survival of *L. monocytogenes*

The widespread presence of *L. monocytogenes* in the environment and its ability to grow at refrigerated temperatures, allows for cross-contamination of RTE products after thermal processing. The International Life Sciences Institute Expert Panel (2005) outlined the five factors that products carrying a high-risk for *Listeria monocytogenes* possess. These characteristics include:

- 1.) Potential for contamination with *L. monocytogenes* in the processing environment.
- 2.) Support growth of *L. monocytogenes* to high numbers.
- 3.) Are ready to eat and do not require reheating prior to consumption.

4.) Require refrigeration.

5.) Are stored for a long period of time.

The environment and composition of RTE meat products serve as an ideal medium for growth of *Listeria monocytogenes*. Key factors such as: temperature, pH (acidity), water activity and salt are extremely important to meat processors as they will have an impact on the overall growth or survival of *Listeria monocytogenes* in meat products. Within the production process, a variety of physico-chemical factors, used either singly or in combination (hurdle technology) can be effective in controlling the survival and growth of *L. monocytogenes* both during processing and in the finished food products (Bell and Kyriakides 2002). An understanding and manipulation of intrinsic and extrinsic factors in meat products is vital in assessing the risk, as well as intervention strategies for control of this virulent pathogen.

Temperature. Growth of *L. monocytogenes* can occur between 0°C to 45°C, with an optimal growth range between 30°C and 37°C (Jay and others 2005). Unlike other foodborne pathogens, *L. monocytogenes* is a psychotrophic bacterium that can survive and grow slowly at refrigerated temperatures (4°C) making it extremely problematic to food processors (Yousef and Lado 2009). This unique ability to grow at low temperatures can be attributed to the fatty acid composition of the bacterium. Membrane lipid fatty acids must be maintained in a liquid-crystalline state to maintain fluidity which allows for optimal membrane structure and function at low temperatures (Suutari and Laakso 1994; Annous and others 1997; Yousef and Lado 2009). Annous and others (1997) found that membranes of *L. monocytogenes*

contain >95% branched-chain fatty acids. When cultures were grown in cold temperatures the adaptation of the fatty acids was two-fold. The adaptation of the membrane through shortening and increase in branching of the fatty acids at cold temperatures prevents tight packing of the membrane phospholipids maintaining fluidity and allowing growth to continue (Yousef and Lado 2009).

It is important to reiterate the fact that refrigeration does not provide protection from growth of this hardy microorganism, and transmission can occur in foods that have been properly stored and refrigerated (McClure and others 1991). An elevated temperature however, causes irreversible damage to *L. monocytogenes* resulting in cell death. Heating *L. monocytogenes* at temperatures above 56°C causes ribosomal damage, protein unfolding and denaturation and consequently enzyme inactivation (Jay and others 2005). Nevertheless, growth and survival may vary depending on the composition of fat and sodium in the meat product (Grau and Vanderlinde 1992; Taromina and Beuchat 2002). Resistance of *L. monocytogenes* to mild heat increases with the food's pH, fat content and salt concentration. While temperatures above optimum will typically have some lethal effect, instances of heat shock response and stress adaptation have been reported, indicating that *L. monocytogenes* may be able to withstand thermal processing treatments. This phenomenon has been found to occur at sublethal temperatures ranging from 43 to 52°C (Farber and Brown 1990; Smith and Marmer 1990). This suggests that if foods containing *L. monocytogenes* are temperature-abused for even short periods, the

organisms will acquire an increased heat tolerance and will require higher inactivation temperatures or longer processing times (Smith and Marmer 1990).

The changes in processing procedures associated with the production of uncured, no-nitrate-or-nitrite-added meats subjects a product to incubation temperatures at approximately 38°C for 1-2 hours, depending upon the type of product produced. The aforementioned studies demonstrate the ability of naturally present *L. monocytogenes* to withstand typical inactivation temperatures when heat shock occurs. The implications of a natural curing system on the impacts of pathogen adaptation via stress response have not yet been established. These studies further emphasize the importance of recognizing all points at which *L. monocytogenes* will be controlled throughout the production process. While it is widely recognized that contamination typically occurs during post processing environment during procedures such as peeling, slicing, and packaging of fully cooked meat products other control points may need to be established in the production process.

Salt (NaCl) Concentration. Salt is a key non-meat ingredient used in the production of processed meats and can have an impact on microbial growth and survival. Unlike many spoilage and pathogenic bacteria *L. monocytogenes* can endure extremely high salt concentrations (Farber and others 2007). *L. monocytogenes* is halotolerant and has been found to survive for 259 days in commercial cheese brine with a 23.8% NaCl concentration (Larson and others 1999). High salt concentrations can decrease the growth rate through lowering of

the water activity (Petran and Zottola 1989). The lag phase was reported to increase from 5 to 8 days when 4% NaCl was added and up to 13 days after 6% salt was added (Cole and others 1990). Nevertheless such salt concentrations would deem products unacceptable to consumers, as they are higher than the levels typically added to processed meat products (Jay and others 2005). This indicates that salt alone is not sufficient to inhibit the growth of *Listeria monocytogenes* in RTE meat products.

Sodium Nitrite. While sodium nitrite is a strong inhibitor of anaerobic, spore forming bacteria such as *Clostridium botulinum* the effects on *L. monocytogenes* are not as profound. Nevertheless, nitrite has been shown to be an important parameter for the survival and growth of *Listeria* as this curing agent slightly inhibits the growth of *L. monocytogenes* (Buchanan and Phillips 1990). Grau and Vandelinde (1990) investigated the growth of *L. monocytogenes* on processed corned beef and ham and they found that residual nitrite played an important role in the overall control of the pathogen as *Listeria monocytogenes* did not grow on ham containing 170ppm residual nitrite at 0°C, but did grow on ham with 11ppm nitrite at 0°C. Sodium nitrite at 200ppm in combination with 5% NaCl, has been shown to inhibit *L. monocytogenes* growth for 40 days at 5°C in vacuum-packaged salmon (Pelroy and others 1994). These results disagree with Glass and Doyle (1989a), who reported that combining 103ppm sodium nitrite and 3.5% sodium chloride in meat did not control growth of *L. monocytogenes* at 32°C in beaker sausage and pepperoni. Similar to the means by which nitrite impacts *C. botulinum*, the mechanism behind

growth suppression of *L. monocytogenes* is unclear (Cammack and others 1999a). However, it is thought to be a result of compounds that are formed during the curing process, including nitric oxide and production of low levels of nitrous acid (Yousef and Lado 2009).

pH (acidity). *Listeria* grows optimally at a pH range between 6-8 (Jay and others 2005), but has been shown to grow at values ranging from 4.0-9.6 (Yousef and Lado 2009). As pH decreases below 6.5 lag phase and generation time increase considerably (Buchanan and Phillips 1990). Lowering of pH can be either inhibitory or fatal to the cell as the growth capability of bacteria is dependent upon the ability to maintain a neutral environment within the cell walls. Typically the cell can maintain the export of H^+ in mildly acidic conditions, and an inhibitory effect occurs. As the pH is lowered, the cell is unable to export the excess H^+ outside of the cytoplasm, and a decrease in intracellular pH is inevitable. The decrease in intracellular pH alters enzymatic activity and may denature proteins resulting in cell death (Buchanan and Phillips 1990; Yousef and Lado 2009). Despite this, *L. monocytogenes* has been found to endure the fermentation or pH reduction process in the production of hard salami (pH 4.4) and continued to survive throughout refrigerated storage (Johnson and others 1988).

Water Activity (a_w). Like most bacteria, *L. monocytogenes* grows best at $a_w \geq 0.97$, but can survive in environments below <0.90 under refrigeration conditions. In fact, *L. monocytogenes* was able to survive in fermented hard salami with water activity ranging between 0.79 to 0.86 for at least 84 days (Johnson and others

1988). Petran and Zottola (1989) performed a study using trypticase soy broth and found that the minimum a_w that permitted growth was 0.92. Further investigations indicated that some strains of *L. monocytogenes* had the ability to grow at a_w levels as low as 0.90 in brain heart infusion broth (Farber and others 1992), which makes *L. monocytogenes* one of two pathogens capable of growing at a_w values <0.93 (Jay and others 2005)

The differences in growth rates can be seen not only across product categories, but also within the same category. In 1989, Glass and Doyle conducted a study to determine the fate of *L. monocytogenes* in processed meat products during storage and found that growth rates were product dependent. Not only were differences seen among different product categories, differences were also seen within the same product category most notably frankfurters (Glass and Doyle 1989b). These results emphasize the importance of understanding intrinsic factors such as pH, a_w , salt concentration and nitrite to better understand what factors are imperative to control or prevent the growth of *L. monocytogenes* in RTE meat products.

Foodborne Listeriosis

Ingestion of contaminated RTE meat products such as deli meats and frankfurters, can result in listeriosis in susceptible populations. As with most foodborne pathogens those populations include the young, elderly, and immune compromised individuals. In addition, pregnant women are about 20 times more likely than other healthy adults to contract listeriosis (Tappero and others 1995).

While the mother is rarely affected, the disease is detrimental to the fetus, as listeriosis can result in spontaneous abortion, still birth or premature birth.

The infective dose of *L. monocytogenes* varies with the strain and with the susceptibility of the victim. From cases contracted through raw or supposedly pasteurized milk, one may safely assume that in susceptible persons, fewer than 1,000 total organisms may cause the disease. Once the bacterium enters the host's monocytes, it becomes blood-borne (septicemic) and can grow. *L. monocytogenes* also has the ability to permit access to the brain and can undergo transplacental migration to the fetus in pregnant women causing stillbirths, preterm labor and even abortions.

Incidence of Foodborne Listeriosis

Currently, *L. monocytogenes* is widely recognized as a significant food safety hazard in the meat and poultry industry. Nevertheless, the history of *L. monocytogenes* and foodborne listeriosis is relatively new when compared to the wide variety of pathogens that have been implicated in foodborne outbreaks for decades. It wasn't until recently that *L. monocytogenes* began to emerge as a serious foodborne pathogen in RTE meat and poultry products. A 1981 outbreak of listeriosis in Nova Scotia involving 41 cases and 18 deaths was epidemiologically linked to the consumption of coleslaw containing cabbage that had been fertilized with sheep manure contaminated with *L. monocytogenes* (Braden and Norton, 2009). Until this time, listeriosis was considered a rare and sporadic human disease.

In the late 1990's, 35 million pounds of frankfurters and deli meats were implicated in a multistate outbreak of listeriosis and caused for examination of retail RTE meat and poultry products. Between January 1994 and October 2006 at least 175 separate recalls were issued for cooked and RTE meat contaminated with *L. monocytogenes* in the United States, including 74 for deli meats, 42 for sausages, 37 for hot dogs and 22 for other products (Farber and others 2007).

Regulations Regarding *L. monocytogenes* in RTE Meat Products

As realization of the detrimental effects that could occur and knowledge of the pathogen's ubiquity, resistance to adverse conditions, mild processing (Farber and others 2007) as well as the ability to of *L. monocytogenes* to survive and proliferate under refrigerated conditions served as grounds for the establishment of a "zero tolerance" policy by the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) in 2003. RTE product is considered adulterated if it contains *L. monocytogenes* or if it comes into direct contact with a food contact surface which is contaminated with *L. monocytogenes* (Code of Federal Regulations 2003).

Listeria monocytogenes can contaminate RTE products that are exposed to the environment after they have undergone a lethality treatment. If a meat processing establishment is producing post-lethality exposed RTE products, *L. monocytogenes* is a hazard that must be controlled through its HACCP (Hazard Analysis Critical Control Point) plan or be prevented in the processing environment through prerequisite programs.

The final rule, published as a result of a multi-state outbreak of listeriosis from contaminated turkey deli meat, established three alternatives to effectively control *L. monocytogenes* in RTE meat products. In order to maintain the sanitary conditions necessary to meet this requirement, an establishment producing post-lethality exposed RTE product must comply with the requirements included in one of the three following alternatives as outlined in 9CFR 430 (Code of Federal Regulations 2003):

- (1) Alternative 1. Application of a post-lethality treatment (which may be an antimicrobial agent) that reduces or eliminates microorganisms on the product **and** an antimicrobial agent or process that suppresses or limits the growth of *L. monocytogenes*.
- (2) Alternative 2. Application of **either** a post-lethality treatment (which may be an antimicrobial agent) that reduces or eliminates microorganisms on the product **or** an antimicrobial agent or process that suppresses or limits growth of *L. monocytogenes*.
- (3) Alternative 3. Use of sanitation measures only.

Despite the stringent regulations in place for *L. monocytogenes* in meat products, adulteration of RTE meat products with this hardy microorganism is still a leading cause of recalls in the meat industry today.

VIII. Control of *L. monocytogenes* in Uncured, No-Nitrate-or-Nitrite-Added (Natural or Organic) RTE Meat Products

The control of *L. monocytogenes* represents a considerable challenge to processors of RTE meat and poultry products especially those that are produced under uncured, natural, or organic methods. Prevention of post processing contamination and reformulation of meat products to inhibit growth have been identified as critical strategies to reduce the risk of listeriosis (International Life Sciences Institute Research Foundation 2005). Unfortunately, processors employing natural, organic or minimally processed methods to produce meat products are at a disadvantage as these typical reformulation options may not be ingredients approved for use in the production of natural and organic meat products.

The use of organic acids such as lactate and diacetate are widely used in the production of conventionally processed meat products to meet alternatives one and two for control of *L. monocytogenes* in RTE meat products. Despite their advantages in suppressing the growth of *L. monocytogenes* throughout extended refrigerated storage, these antimicrobials are considered to be preservatives. As a result, they are not permitted for use in natural or organic meat products. Interestingly, this was not always the case. In 2005 the USDA-FSIS made an amendment to the Food Standards and Labeling Policy Book to allow sodium lactate usage in natural and organic products. Concerns with the amendment arose, and in October of 2006 the USDA-FSIS received petition from Hormel Foods which indicated that the 2005 change was inconsistent with the original policy and creates

confusion regarding whether a meat or poultry product bearing a natural claim may contain chemical preservatives and synthetic ingredients (Minerich 2006). Addition of sodium lactate was allowed only at reduced levels for flavoring purposes, up to 2% of the product formulation as opposed to levels at 4.8% used for antimicrobial purposes (USDA 2005), yet Hormel felt that the exemption was inconsistent with the longstanding natural policies, which denied natural claims for products that contained chemical preservatives.

Chemical preservatives, as defined by 21 CFR 101.22 are any chemicals that when added to food tends to prevent or retard deterioration thereof. Common natural preservatives including salt, sugars, vinegars, spices or oils extracted from spices and substances added to food by direct exposure to wood smoke are exempt from this definition (Code of Federal Regulations 2008a).

Sodium lactate is an approved generally recognized as safe substance (GRAS) that is permitted for use at levels up to 4.8% of formulation weight in meat and poultry products to inhibit microbial growth, and would follow the definition of a chemical preservative (Code of Federal Regulations 2008b). Sodium lactate is also listed as part of a class of substances that serve as flavoring agents, protectors and developers as further outlined in 9 CFR 424.21. When utilized as an emulsifier, flavoring agent, flavor enhancer, humectants or pH control agent the amount added is not to exceed 2 percent of the formulation (Code of Federal Regulations 2008b). These reduced levels for flavoring purposes are those that were outlined in the 2005 edition of the Food Standards and Labeling Policy Book in which sodium

lactates (from a corn source) were approved for use in products carrying natural claims. While the levels are remarkably less than those that are typically used for antimicrobial and preservation purposes, Hormel argued that the reduced levels for flavoring would still have a preservative effect. These arguments were substantiated by US Patent No 4,798,729 held by Oscar Mayer which claim a delay in the growth of *Clostridium botulinum* with lactate levels as low as 1% (Anders and others 1989). In research studies conducted by Maas, Glass and Doyle (1989), it was found that sodium lactate delayed toxin production by *Clostridium botulinum* in a model system using comminuted turkey breast. Results indicated that as the level of sodium lactate was increased from 2.0 to 3.5%, the production of toxin by *C. botulinum* was delayed. Ground samples containing 2.0% sodium lactate became toxic at 4 to 5 days while samples containing 3.5% did not become toxic until days 7 to 8. However, authors indicated that a minimum threshold level of lactate was required for delay of botulinal toxin production, as no delay was observed with a treatment containing 1.92% (Maas and others 1989). These results indicated while sodium lactate can inhibit microorganisms, the antimicrobial impact is concentration dependent. The levels approved for flavoring purposes, as well the addition to natural products in accordance with the 2005 version of the Food Standards and Labeling Policy Book indicate that 2% would be secondary to its flavoring attributes making it a dual purpose ingredient.

In addition to concerns with the antimicrobial impact of sodium lactate in natural products, the petition also points out that the process by which sodium

lactate is manufactured is more than minimally processed (Minerich 2006). Commercially available sodium lactate is produced by the fermentation of sucrose, originating from sugar cane or beet or dextrose originating from corn. While fermentation is recognized as minimal processing, subsequent steps in the production process are not. The lactic acid is then combined with a chemical, sodium hydroxide allowing the sodium to act as a carrier of the lactic acid. As a result, the process by which sodium lactate is produced undergoes chemical hydrolysis after a fermentation process, which would indicate that the product itself is more than minimally processed. The use of such a flavoring that has undergone more than minimal processing would, in general, mean that a product which contains the ingredient could not be labeled as natural (USDA 2005).

As a result of the controversy of natural and the disagreements about the policy modification, the USDA published a notice in December of 2006 to remove the reference to sodium lactate from the 2005 Foods Standards and Labeling Policy Book. Until the agency finalizes rulemaking on “natural,” “natural” claims for meat and poultry products which contain sodium lactate in the formulation will be considered on a case-by-case basis and will examine factors including the level of usage, as well as the claimed and actual effect that sodium lactate is having on the product (Food Safety and Inspection Service 2006).

Similar debates regarding lactate usage were also present in the organic meat and poultry sector. Inconsistencies in the approval process were uncovered when it was discovered that the USDA had improperly approved ingredients that are

not on the National List of Allowed and Prohibited Materials in Organic products. This transpired in 2004 after Applegate Farms, a manufacturer of organic processed meat products, submitted a petition to allow the use of sodium and potassium lactate as preservatives in organic processed meat products. Neither of these substances is on the National List, and neither has been reviewed by the National Organics Standards Board (NOSB). According to the Organic Food Production Act (OFPA) of 1990, all petitioned materials must be approved by the NOSB prior to use in organic products. Nevertheless, the USDA ruled that sodium and potassium lactates could be used without review because they are manufactured from substances that are on the National List.

The amount of controversy surrounding the use of lactates, and reduction in hurdle technologies such as nitrite, in natural and organic products brings about apprehensions on the overall safety of uncured, no-nitrate-or-nitrite-added products, especially with the known hazard associated with *Listeria monocytogenes*. Currently, there are a limited number of non-meat ingredients available to processors wishing to products uncured, no-nitrate-or-nitrite-added meat products that meet the regulatory standards for alternatives one or two. With the ongoing concerns with control of *L. monocytogenes*, and the hazard that it poses on susceptible populations, organic and natural antimicrobial solutions need to be further investigated.

IX. Summary of Literature

Uncured, no-nitrate-or-nitrite-added processed meat products have become increasingly popular with a multitude of consumers. Such consumers believe these products are safer, healthier, and more wholesome than conventionally processed foods due to the ongoing negative perceptions of nitrite. While these products are labeled as “uncured” they do in fact contain nitrites that are produced from naturally occurring nitrates found in vegetables and through the addition of a nitrite reducing starter culture.

The complex reactions that nitrite undergoes upon the addition to a meat system is still not fully understood, and natural curing systems add greater confusion. While nitrite levels can provide typical cured meat characteristics, these levels may not be sufficient to serve as a barrier for microbial control. The addition of sodium nitrite to meat products has served as a powerful protectant against foodborne pathogens for centuries and is synergistic with other non-meat ingredients that dramatically improve the safety of ready-to-eat meat products. Modifications in non-meat ingredients and processing procedures to meet the requirements of natural and organic may have negative impacts on the safety of meat products. Lower levels of nitrite and removal of other commonly used preservatives place consumers at risk as the impacts of these new processing technologies on food safety are still unknown.

REFERENCES

- [AMI] American Meat Insititute. 2003. Questions and answers: sodium nitrite use and safety. AMI Fact Sheet. Washington, DC.
- [AMI] American Meat Insititute. 2008. Alleged hot dog and cancer connection: the truth about vegan group's cancer claims. AMI Update. Washington, DC.
- Aberle ED, Forrest JC, Gerrard DE, Mills EW, Hedrick HB, Judge MD & Merkel RA. 2001. Principles of meat science, 4th ed. Dubuque, Iowa: Kendall/Hunt.
- Ahn D & Maurer AJ. 1989. Effects of added nitrite, sodium chloride, and phosphate on color, nitrosoheme pigment, total pigment, and residual nitrite in oven-roasted turkey breast. *Poult Sci* 68:100-106.
- Ahn HJ, Kim JH, Jo C, Lee CH & Byun MW. 2002. Reduction of carcinogenic N-nitrosamines and residual nitrite in model system sausage by irradiation. *J Food Sci* 67:1370-1373.
- Anders RJ, Cervený JG & Milkowski AL, inventors; Oscar Mayer Foods Corporation, assignee. Jan 17, 1989. Method for delaying *Clostridium botulinum* growth in fish and poultry. United States patent 4,798,729.
- Annous BA, Becker LA, Bayles DO, Labeda DP & Wilkinson BJ. 1997. Critical role of anteiso-C-15:0 fatty acid in the growth of *Listeria monocytogenes* at low temperatures. *Appl Environ Microbiol* 63(10):3887-3894.
- Archer DL. 2002. Evidence that ingested nitrate and nitrite are beneficial to health. *J Food Prot* 65:872-875.
- Bacus JN. 1984. Utilization of microorganisms in meat processing : a handbook for meat plant operators. Letchworth, Hertfordshire, England New York: Research Studies Press ;Wiley.
- Bacus JN. 2006. Natural ingredients for cured and smoked meats. Proceedings of the 59th Annual American Meat Science Association Reciprocal Meat Conferance. Champaign-Urbana, Illinois. P 77-78.
- Bednar C & Kies C. 1994. Nitrate and vitamin-C from fruits and vegetables - impact of intake variations on nitrate and nitrite excretions of humans. *Plant Foods Hum Nutr* 45(1):71-80.

- Bell C & Kyriakides A. 2002. *Listeria monocytogenes*. In: Blackburn CW and McClure PJ, editors. Foodborne pathogens hazards, risk analysis and control. 2nd ed. Boca Raton, FL: CRC Press P 337-362.
- Boerema JA, Broda DM & Werner Klinth J. 2004. Microbiological safety of meat | *Clostridium botulinum*. In: Dikeman M, Jensen W, Devine C, editors. Encyclopedia of meat sciences. Oxford: Elsevier. P 786-793.
- Braden C & Norton D. 2009. Foodborne listeriosis. In: Ryser ET & Marth, EH, editors. *Listeria*, listeriosis, and food safety. 3rd ed. Boca Raton, FL: CRC Press. P 308-349.
- Buchanan RL & Phillips JG. 1990. Response-surface model for predicting the effects of temperature, pH, sodium-chloride content, sodium-nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J Food Prot 53(5):370-376.
- Buchrieser C & Rocourt J. 2009. The genus *Listeria* and *Listeria monocytogenes*. In: Ryser ET & Marth EH, editors. *Listeria*, listeriosis, and food safety. 3rd ed. Boca Raton, FL: CRC Press. P 1-12.
- Buncic S, Avery SM & Werner Klinth J. 2004. Microbiological safety of meat | *Listeria monocytogenes*. In: Dikeman M, Jensen W, Devine C, editors. Encyclopedia of meat sciences Oxford: Elsevier. P 804-814.
- Cammack R, Joannou CL, Cui XY, Martinez CT, Maraj SR & Hughes MN. 1999. Nitrite and nitrosyl compounds in food preservation. Biochim Biophys Acta 1411(2-3): 475-488.
- Carruthers A & Carruthers J. 2005. Botulinum toxin type A. J Am Acad Dermatol 53(2):284-290.
- Cassens RG, Greaser M, Ito T & Lee M. 1979. Reactions of nitrite in meat. Food Technol 33(7):46-57.
- Cassens RG. 1995. Use of sodium nitrite in cured meats today. Food Technol 49:72-80, 115.
- Cassens RG. 1997a. Composition and safety of cured meats in the USA. Food Chem 59(4):561-566.
- Cassens RG. 1997b. Residual nitrite in cured meat. Food Technol 51(2):53-55.

- Cho E, Smith-Warner SA 2004. Meat and fat intake and colorectal cancer risk: A pooled analysis of 14 prospective studies. AACR Meeting Abstracts, 2004. P 113.
- Cho IC & Bratzler LJ. 1970. Effect of sodium nitrite on flavor of cured pork. J Food Sci 35:668-670.
- Christiansen LN, Johnston RW, Kautter DA, Howard JW & Aunan WJ. 1973. Effect of nitrite and nitrate on toxin production by *Clostridium botulinum* and on nitrosamine formation in perishable canned comminuted cured meat. Appl Environ Microbiol 25(3):357-362.
- Code of Federal Regulations. 2003. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products; Final Rule. 9CFR 430. Washington, DC: U.S. Government Printing Office.
- Code of Federal Regulations. 2007a. Interpretation and statement of labeling policy for cured products; special labeling requirements concerning nitrate and nitrite. 9CFR 317.17. Washington, DC: U.S. Government Printing Office.
- Code of Federal Regulations. 2007b. Products and nitrates and nitrites. 9CFR 319.2. Washington, DC: U.S. Government Printing Office.
- Code of Federal Regulations. 2008a. Foods; labeling of spices, flavorings, colorings and chemical preservatives. 21CFR 101.22. Washington, DC: U.S. Government Printing Office.
- Code of Federal Regulations. 2008b. Preparations and processing ingredients. 9 CFR 424.21 Subpart C-Food Ingredients and Sources of Irradiation. Washington, DC: U.S. Government Printing Office.
- Cole MB, Jones MV & Holyoak C. 1990. The effect of pH, salt concentration and temperature on the survival and growth of *Listeria monocytogenes*. J Appl Microbiol 69(1):63-72.
- Dangour AD, Dodhia SK, Hayter A, Allen E, Lock K & Uauy R. 2009. Nutritional quality of organic foods: a systematic review. Am J Clin Nutr 90(6): 1701-1707.
- Draudt HN & Deatherage FE. 1956. Studies on the chemistry of cured meat pigment fading. J Food Sci 21(1):122-132.
- Farber JM & Brown BE. 1990. Effect of prior heat shock on heat resistance of *Listeria monocytogenes* in meat. Lett Appl Microbiol 56(6):1584-1587.

- Farber JM, Coates F & Daley E. 1992. Minimum water activity requirements for the growth of *Listeria monocytogenes*. Lett Appl Microbiol 15(3):103-105.
- Farber JM, Pagotto F & Scherf C. 2007. Incidence and behavior of *Listeria monocytogenes* in meat products. In: Ryser ET & Marth EH, editors. *Listeria*, listeriosis, and food safety. 3rd ed. Boca Raton, FL: CRC Press. P 503-570.
- Feiner G. 2006. Meat products handbook : practical science and technology. Boca Raton Cambridge, England: CRC Press ; Woodhead Pub.
- Fernandez PS, Baranyi J & Peck MW. 2001. A predictive model of growth from spores of non-proteolytic *Clostridium botulinum* in the presence of different CO₂ concentrations as influenced by chill temperature, pH and NaCl. Food Microbiol 18(4):453-461.
- Food and Drug Administration, Center for Food Safety and Applied Nutrition, United States Department of Agriculture, Food Safety and Inspection Service & Centers for Disease Control and Prevention. 2003. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Washington, DC: U.S. Government Printing Office.
- Food Safety and Inspection Service. 2006. Product labeling: definition of the term "natural". Washington, DC. USA: Federal Register. P 70503-70504.
- Fooladi MH, Pearson AM, Coleman TH & Merkel RA. 1979. The role of nitrite in preventing development of warmed-over flavour. Food Chem 4(4):283-292.
- Fox JB. 1966. Chemistry of meat pigments. J Agri Food Chem 14(3):207-210.
- Freybler LA, Gray JI, Asghar A, Booren AM, Pearson AM & Buckley DJ. 1993. Nitrite stabilization of lipids in cured pork. Meat Sci 33(1):85-96.
- Gellin BG, Broome CV, Bibb WF, Weaver RE, Gaventa S, Mascola L & Listeriosis Study Group. 1991. The epidemiology of listeriosis in the United States-1986. Am J Epidemiol 133(4):392-401.
- Glass KA & Doyle MP. 1989a. Fate and thermal inactivation of *Listeria monocytogenes* in beaker sausage and pepperoni. J Food Prot 52:226-231.
- Glass KA & Doyle MP. 1989b. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. Appl Environ Microbiol 55(6):1565-1569.

- Grau FH & Vanderlinde PB. 1990. Growth of *Listeria monocytogenes* on vacuum-packaged beef. J Food Prot 53(9):739-741.
- Greene BE & Price LG. 1975. Oxidation-induced color and flavor changes in meat. J Agri Food Chem 23(2):164-167.
- Hill MJ. 1991. Nitrates and nitrites in food and water. West Sussex, England: Ellis Horwood Limited.
- Hunt MC & Hedrick HB. 1977. Profile of fiber types and related properties of 5 bovine muscles. J Food Sci 42(2):513-517.
- Igene JO, Yamauchi K, Pearson AM & Gray JL. 1985. Mechanisms by which nitrite inhibits the development of warmed-over flavor (WOF) in cured meat. Food Chem 18:1-18.
- International Life Sciences Institute Research Foundation. 2005. Achieving continuous improvement in reductions in foodborne listeriosis-a risk-based approach. J Food Prot 68:1932-1994.
- Jay JM, Loessner MJ & Golden DA. 2005. Modern food microbiology. 7th ed. New York, NY: Springer Science and Business Media LLC.
- Johnson JL, Doyle MP, Cassens RG & Schoeni JL. 1988. Fate of *Listeria monocytogenes* in tissues of experimentally infected cattle and in hard salami. Appl Environ Microbiol 54(2):497-501.
- Larson AE, Johnson EA & Nelson JH. 1999. Survival of *Listeria monocytogenes* in commercial cheese brines. J Dairy Sci 82(9):1860-1868.
- Lee K & Shimakoa JE. 1984. Forms of iron in meats cured with nitrite and erythorbate. J Food Sci 49:284-285.
- Lee SH & Cassens RG. 1976. Nitrite binding sites on myoglobin. J Food Sci 41:969-970.
- Lee SH, Cassens RG & Fennema OR. 1976. Effect of muscle type on residual nitrite in cured meat. J Food Sci 41:100-101.
- Lin HS & Sebranek JG. 1979. Effect of sodium nitrite concentration and packaging conditions on color stability and rancidity development in sliced bologna. J Food Sci 44(5):1451-1454.

- Lövenklev M, Artin I, Hagberg O, Borch E, Holst E & Rådstöm P. 2004. Quantitative interaction effects of carbon dioxide, sodium chloride, and sodium nitrite on neurotoxin gene expression in nonproteolytic *Clostridium botulinum* type B. *Appl Environ Microb* 70: 2928-2934.
- Maas MR, Glass KA & Doyle MP. 1989. Sodium lactate delays toxin production by *Clostridium botulinum* in cook-in-bag turkey products. *Appl Environ Microbiol* 55(9):2226-2229.
- MacDonald B, Gray JI & Gibbins LN. 1980. Role of nitrite in cured meat flavor: antioxidant role of nitrite. *J Food Sci* 45(4):893-897.
- Mancini RA & Hunt MC. 2005. Current research in meat color. *Meat Sci* 71(1):100-121.
- McClure PJ, Kelly TM & Roberts TA. 1991. The effects of temperature, pH, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. *Int J Food Microbiol* 14(1):77-91.
- Mead PS, Slutsker L, Griffin PM & Tauxe RV. 1999. Food-related illness and death in the United States. *Emerg Infect Dis* 5(6):841-842.
- Minerich PL. 2006. Petition for the issuance of a rule regarding natural label claims. Hormel Foods Corporate Services. Austin, MN.
- Mintel. 2009. Consumers sticking with organic options in down economy. Mintel Oxygen Reports. Mintel International Group Ltd. Chicago, IL.
- Møller JKS & Skibsted LH. 2002. Nitric oxide and myoglobins. *Chem Rev* 102:1167-1178.
- Murray EGD, Webb RA & Swann MBR. 1926. A disease of rabbits characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp.). *J Pathol Bacteriol* 29(4):407-439.
- National Academy of Sciences. 1981. The health effects of nitrate, nitrite and n-nitroso compounds. Washington D.C: National Academy Press.
- Neubauer H & Götz F. 1996. Physiology and interaction of nitrate and nitrite reduction in *Staphylococcus carnosus*. *J Bacteriol* 178(7):2005-2009.
- Payne MJ. 1990. Interactions of iron-thiol-nitrosyl compounds with the phosphoroclastic system of *Clostridium sporogenes*. *Microbiol* 136(10):2077.

- Pearson AM & Gillett TA. 1996. Processed meats. 3rd ed. New York, NY: Chapman and Hall.
- Pearson AM, Love JD, Shorland FB, C.O. Chichester EMM & Stewart GF. 1977. "Warmed-over" flavor in meat, poultry, and fish. Adv Food Res P 1-74.
- Pegg RB & Shahidi F. 1997. Unraveling the chemical identity of meat pigments. Crit Rev Food Sci Nutr 37(6):561-589.
- Pegg RB & Shahidi F. 2000. Nitrite curing of meat : the n-nitrosamine problem and nitrite alternatives. Trumbull, Conn.: Food & Nutrition Press.
- Pelroy G, Peterson M, Paranjpye R, Almond J & Eklund M. 1994. Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium nitrite and packaging method. J Food Prot 57:114-119.
- Peters JM, Preston-Martin S, London SJ, Bowman JD, Buckley JD & Thomas DC. 1994. Processed meats and risk of childhood leukemia. Cancer Causes Control 5:195-202.
- Petran RL & Zottola EA. 1989. A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. J Food Sci 54(2):458-460.
- Pierson M & Smoot L. 1982. Nitrite, nitrite alternatives, and the control of *Clostridium botulinum* in cured meats. Crit Rev Food Sci Nutr 17(2):141-187.
- Pirie JH. 1940. *Listeria* - Change of name for a genus of bacteria. Nature 145:264-264.
- Price JF & Schweigert BS. 1987. The science of meat and meat products. 3rd ed. Westport, CT: Food and Nutrition Press, Inc.
- Prusa KJ & Kregel KK. 1985. Effect of muscle type and sodium tripolyphosphate on residual nitrite, pH, color, and instron measurements of turkey frankfurters. Poult Sci 64(11):2165-2170.
- Reddy D, Lancaster JR & Cornforth DP. 1983. Nitrite inhibition of *Clostridium botulinum*: electron spin resonance detection of iron-nitric oxide complexes. Science 221(4612):769-770.
- Richardson WD. 1907. The occurrence of nitrates in vegetable foods, in cured meats and elsewhere. J Am Chem Soc 29(12):1757-1767.
- Romans JR, Costello WJ, Carlson WC, Greaser ML, Jones, KW. 2001. The meat we eat, 14th ed. Danville, Ill.: Interstate Publishers.

- Rust RE & Olson DG. 1973. Meat curing principles and modern practice. Kansas City, MO: Koch Supplies Inc.
- Scharff RL. 2010. Health-related costs from foodborne illness in the United States. Produce Safety Project at Georgetown University. P 27.
- Sebranek JG. 1979. Advances in the technology of nitrite use and consideration of alternatives. *Food Technol* 33(7):58-63, 93.
- Sebranek JG. 1980. Nitrates, nitrites, and the chemistry of meat color. *Proceedings Second Annual Sausage and Processed Meats Short Course*: P 8-14.
- Sebranek JG & Bacus JN. 2007. Cured meat products without direct addition of nitrate or nitrite: what are the issues? *Meat Sci* 77(1):136-147.
- Sebranek JG & Fox JB, Jr. 1985. A review of nitrite and chloride chemistry: interactions and implications for cured meats. *J Sci Food Agri* 36:1169-1182.
- Sebranek JG, Schroder BG, Rust RE & Topel DG. 1977. Influence of sodium erythorbate on color development, flavor, and overall acceptability of frankfurters cured with reduced levels of sodium nitrite. *J Food Sci* 42:1120-1121.
- Sen NP, Miles WF, Donaldson B, Panalaks T & Iyengar JR. 1973. Formation of nitrosamines in a meat curing mixture. *Nature* 245(5420):104-105.
- Shahidi F. 1998. The flavor of meat, meat products and seafoods. 2nd ed. London, UK: Blackie academic and Professional P 290-317.
- Simon SS, Ellis DE, MacDonald BD, Miller DG, Waldman RC & Westerberg DO. 1973. Influence of nitrite and nitrate curing ingredients on quality of packaged frankfurters. *J Food Sci* 38(6):919-920.
- Sindelar JJ. 2006. Investigating uncured no-nitrate-or-nitrite-added processed meat products. Ph.D. Dissertation, Iowa State University. Ames, IA. p 247.
- Sindelar JJ, Cordray JC, Olson DG, Sebranek JG & Love JA. 2007a. Investigating quality attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial hams, bacons, and frankfurters. *J Food Sci* 72(8):551-559.
- Sindelar JJ, Cordray JC, Sebranek JG, Love JA & Ahn DU. 2007b. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. *J of Food Sci* 72(6):388-395.

- Sindelar JJ, Cordray JC, Sebranek JG, Love JA & Ahn DU. 2007c. Effects of vegetable juice powder concentration and storage time on some chemical and sensory quality attributes of uncured, emulsified cooked sausages. *J Food Sci* 72(5):324-332.
- Smith JL & Marmer BS. 1990. Temperature shift effects on injury and death in *Listeria monocytogenes* Scott A. *J Food Safety* 11(2):73-80.
- Suutari M & Laakso S. 1994. Microbial fatty acids and thermal adaptation. *Crit Rev Microbiol* 20(4):285-328.
- Talon R, Walter D, Chartier S, Barrière C & Montel MC. 1999. Effect of nitrate and incubation conditions on the production of catalase and nitrate reductase by staphylococci. *Int J Food Micro* 52:47-56.
- Tarladgis BG. 1962. Interpretation of the spectra of meat pigments II. - cured meats. The mechanism of colour fading. *J Sci Food Agri* 13(9):485-491.
- Tompkin RB. 1995. Nitrite. In: Davidson PM, Sofos JN & Brannen AL, editors. *Antimicrobials in food*. 3rd ed. Boca Raton, FL: CRC Press, Taylor & Francis Group 171-221.
- Townsend WE & Olson DG. 1987. Cured meats and cured meat products processing. In: Price JF & Schweigert, BS, editors. *The science of meat and meat products*. 3rd ed. Westport, CT: Food & Nutrition Press, Inc.
- USDA. 1995. Processing inspector's calculations handbook (FSIS Directive 7620.3). Washington, DC: U.S. Government Printing Office.
- USDA. 2005. Food standards and labeling policy book (FSIS Directive 7620.3). Washington, DC: U.S. Government Printing Office.
- Wasserman AE & Talley F. 1972. The effect of sodium nitrite on the flavor of frankfurters. *J Food Sci* 37(4):536-538.
- White JW. 1975. Relative significance of dietary sources of nitrate and nitrite. *J Agri Food Chem* 23(5):886-891.
- Wiedmann M & Sauters B. 2009. Ecology of *Listeria* Species and *L. monocytogenes* in the Natural Environment. In: Ryser ET & Marth, EH, editors. *Listeria, listeriosis, and food safety*. 3rd ed. Boca Raton, FL: CRC Press. P 21-44.

Winter CK & Davis SF. 2006. Organic foods. J Food Sci 71(9):117-124.

Wolff IA & Wasserman AE. 1972. Nitrates, nitrites, and nitrosamines. Science 177(4043):15-19.

Yousef A & Lado B. 2009. Characteristics of *Listeria monocytogenes* Important to Food Processors.. In: Ryser ET & Marth, EH, editors. *Listeria*, listeriosis, and food safety. 3rd ed. Boca Raton, FL: CRC Press. P 158-198.

CHAPTER 3. INVESTIGATING THE CONTROL OF *LISTERIA MONOCYTOGENES* ON UNCURED, NO-NITRATE-OR-NITRITE-ADDED PROCESSED MEATS

A paper to be submitted to the Journal of Food Science

Kohl D. Schrader, Joseph C. Cordray, Joseph G. Sebranek, Dennis G. Olson,
Kenneth J. Prusa and Aubrey F. Mendonca

Abstract

Sodium nitrite, utilized in the production of cured meat products, is not permitted for direct addition during the production of natural and organic processed meats. Additionally, common additives such as sodium lactate, used for anti-listerial control, are only tolerated for use at reduced levels for flavoring purposes. As a result, meat processors have begun to employ alternative curing methods utilizing naturally occurring nitrates and nitrites found in vegetables to produce products labeled as uncured, no-nitrate-or-nitrite-added. The objective of this study was to determine if an increased risk for growth of *Listeria monocytogenes* (*Lm*) is associated with the ingredients and procedures used for natural and organic processed meats. Ten brands of commercially available frankfurters [2 conventionally cured controls (A,B), 3 alternatively cured including lactate (E,F,G), 4 alternatively cured without lactate (C,D,H,J) and 1 truly uncured (no addition of nitrite

or lactate; I)] and eight brands of commercially available deli hams [3 conventionally cured controls (A,B,C), 2 alternatively cured including lactate (E,G), 3 alternatively cured without lactate (D,F,H)] were purchased from retail outlets and analyzed for chemical [pH, salt, residual nitrite, water activity (a_w)] attributes which impact microbial growth. Microbial challenge studies with *Lm* were also performed. Measurements of residual nitrate, residual nitrite, sodium chloride, pH and a_w indicated that the majority of the products were similar to the controls for their respective product category. Significant differences ($P<0.05$) in growth of *Lm* existed among uncured, no-nitrate-or-nitrite-added when compared to conventionally processed controls. Rate of growth over the first 14 days was greatest ($P<0.05$) for uncured frankfurter brands, truly uncured brands and alternatively cured brands without lactate. Frankfurter brands containing lactate had growth rates that were similar ($P>0.05$) to both controls. No significant differences were observed between the growth rates of uncured, no-nitrate-or-nitrite-added hams which contained lactate and those which did not. However, these uncured products resulted in significantly higher ($P<0.05$) populations of *Lm* when compared to conventionally processed controls. These results indicate a greater risk of *Lm* growth in alternatively cured meat products and additional intervention strategies to reduce the risk for *Lm* in natural and organic ready-to-eat meats are warranted.

Introduction

Many consumers have recently become infatuated with the concept that minimally processed, preservative free meat products marketed as natural or

organic are more nutritious and “safer” than conventionally processed products. Sodium nitrite (NaNO_2) is a common preservative found in processed meat products and has been under scrutiny by such consumers for decades. Nitrite is classified as a preservative and color fixative according to the Code of Federal Regulations 21 CFR 172.15. Consequently, it is prohibited for direct addition into natural and organic meat products (Bacus 2006). NaNO_2 is a unique, irreplaceable ingredient that is well known for the development of cured meat color and flavor in products such as ham and frankfurters (Fox 1966b; MacDonald and others 1980). While the qualitative benefits of cured color and flavor are quite noticeable for consumers to recognize and appreciate, the ability of nitrite to inhibit the outgrowth of certain bacteria is far more important and valuable. Nitrite is a bacteriostatic agent that inhibits the growth of both spoilage and pathogenic microorganisms. Nitrite is a strong inhibitor of anaerobic bacteria including *Clostridium botulinum* and has been shown to assist in the control of *Listeria monocytogenes* (Tompkin 1995).

Despite its proven track record for food safety, processors are utilizing alternative methods to meet the increased demands for preservative free meat products by natural and organic consumers. These alternative curing methods utilize naturally occurring nitrates and nitrites found in vegetables and sea salts to result in processed meats that demonstrate typical cured meat properties including color and flavor (Sindelar and others 2007a). While finished meat products possess typical cured meat properties they have also been found to result in greater variation of these properties than observed for conventionally cured meats (Sindelar 2006;

Sebranek and Bacus 2007). While nitrite levels can provide typical cured meat characteristics, these levels may not be sufficient to serve as a barrier for microbial control. The direct addition of sodium nitrite to meat products has served as a powerful hurdle technology against foodborne pathogens for centuries. The implication of natural nitrate and nitrite sources on the safety of uncured, no-nitrate-or-nitrite-added meat products is not known. Consequently, the changes in non-meat ingredients and processing procedures used for natural and organic processed meats are cause for examination to determine if significant foodborne hazards exist.

Materials and Methods

Experimental Design and Data Analysis

The experimental design was a randomized complete block design using a mixed effects model. Statistical analysis was performed for all measurements using the Statistical Analysis System (SAS Institute 2008). The model included the random main effect of replication and the fixed main effect of Brand (A-J and A-H). Brands A, B for frankfurters and Brands A, B, C for deli ham = nitrite added controls. Brands C-J and D-H = uncured, no-nitrate-or-nitrite-added for frankfurters and hams respectively. The model also included the fixed main effects of treatment, replication, day, and the interaction of treatment x day. Within the main factorial design was a split plot for measurements over time. The split plot contained 6 sampling periods (day 0, 7, 14, 21, 28, 35). The random effect was the interaction of treatment x replication. Contrast comparisons and correlations were also performed to determine factors that were contributing to microbial growth.

The significant main effect means for all experiments were separated and least significant differences were found using the Tukey-Kramer multiple pairwise comparison method. Significance was determined at $P < 0.05$. This experiment was replicated twice.

Product Procurement

Ten brands of commercially available frankfurters (eight, uncured, no-nitrate-or-nitrite-added, and two nitrite added controls) and eight brands of commercially available ham (five, uncured, no-nitrate-or-nitrite-added and three nitrite-added controls) were obtained from retail outlets and transported under refrigerated conditions to the Iowa State University Meat Laboratory (Ames, Iowa, U.S.A.) on two separate occasions (replications). Selection of each brand was based solely on sell-by dates indicated on the package. Ham Brands A, B, and C and frankfurter Brands A and B were selected as a standard for both quality and safety in their respective categories and all included the antimicrobials lactate and/or diacetate in the formulation, representing typical antilisterial control measures. Upon arrival at the Iowa State University Meat Laboratory, brands were assigned codes (A-J for frankfurters and A-H for ham) and stored under refrigeration (0-2°C).

Frankfurters from each brand were randomly selected for microbial analysis and were set aside and later dipped in boiling water as described in (Radin and others 2006). This method reduced the amount of lactic acid spoilage bacteria present on the surface to allow for more consistent enumeration of *Listeria monocytogenes*. Following cooling to 4°C, dipped frankfurters were packaged and

transferred to the Iowa State University Food Safety Research Laboratory for subsequent challenge studies. All frankfurters used for chemical and microbial analysis were vacuum packaged (Multivac Model A6800 vacuum packager, Multivac Inc., Kansas City, Mo., U.S.A.) using barrier bags (Cryovac B540, Cryovac Sealed Air Corp., Duncan, S.C., U.S.A.) that had an O₂ transmission rate of 3-6 cc/m² per 24 h at 1 atm, 4.4 °C, and 0% RH, and a water vapor transmission rate of 0.5-0.6 g/645 cm² per 24 h and 100% RH.

Un sliced ham treatments were sliced to a comparable thickness of the remaining presliced treatments used in the study. A sufficient number of slices were reserved for inoculation and analysis in the Food Safety Research Laboratory. Ham slices for chemical analysis were packaged (Multivac Model A6800 vacuum packager, Multivac Inc., Kansas City, Mo., U.S.A.) using the Cryovac B540 packaging film that was utilized in the frankfurter study.

Water Activity

Available moisture was determined using a handheld Pa_wkit water activity meter (Decagon Devices Inc., Pullman, Wa., U.S.A.). Measurements were obtained once weekly throughout the 6 week sampling period and performed in duplicate.

Sodium Chloride Analysis

Sodium chloride content was determined using the Quantab Method as described by Sebranek and others (1990). Ground samples were analyzed in

duplicate on day 0 using titrating strips (Quantab Chloride Tirator, Environmental Test Systems, Inc., Elkhart, Ind., U.S.A.).

pH Determination

The pH of ham and frankfurter samples was determined by grinding the samples and then directly measuring pH in the ground sample with a pH/ion meter (Accumet 925: Fisher Scientific, Fair Lawn, N.J., U.S.A) equipped with an attached electrode (Accumet Flat Surface Epoxy Body Ag/AgCl combination Electrode Model 13-620-289, Fisher Scientific, Fair Lawn, N.J., U.S.A) calibrated with phosphate buffers 4.0 and 7.0. For each brand measurements were made in duplicate.

Residual Nitrite Analysis

Residual nitrite was determined by utilizing the AOAC method ([AOAC] Association of Official Analytical Chemists, 1990). The same finely chopped/ground samples that were used for pH determination were used for the residual nitrite measurement. All assays were executed in duplicate.

Residual Nitrate Analysis

Methods for residual nitrate analysis were modifications of Ahn and Maurer (1987). Five grams of meat product samples were weighed in a 50 ml test tube and homogenized with 20 ml of distilled, de-ionized water using a Polytron homogenizer (Type PT 10/35, Brinkman Instruments Inc., Westbury, N.Y., U.S.A.) at high speed for 10 seconds. The homogenate was then heated for 1 hour in an 80° C water bath. Following cooling in cold water for 10 minutes, 2.5 ml of the homogenate was

transferred to a disposable test tube (16 x 100 mm). Carrez II (dissolve 10.6 g potassium ferrocyanide in 100 ml of distilled, de-ionized water) and Carrez I (dissolve 23.8 g of zinc acetate in 50 ml of distilled, de-ionized water, then add 3 ml of glacial acetic acid and dilute with distilled, de-ionized water to 100 ml) reagents were added (0.1 ml each) to precipitate proteins. The solution was diluted with 2.3 ml of distilled, de-ionized water and mixed thoroughly. Following precipitation, the supernatant was centrifuged at 10,000 x g for 20 minutes and the clear supernatant was used for nitrate analysis. Analysis was performed using high performance liquid chromatography (Agilent 1100 Series HPLC System, Agilent Technologies, Wilmington, Del., U.S.A.) with and Agilent Zorbax SAX column (analytical 4.6 x 150 mm, 5-micron) (Agilent, Wilmington, Del., U.S.A.). The elution buffer was 15mM phosphate buffer, pH of 2.35, with isocratic elution. The flow rate was 1.0 ml/min and the sample volume was 25 μ L. A 210 nm wavelength was utilized and the area of the nitrate peak was used to calculate nitrate concentration using a nitrate standard curve. Results were reported in parts per million (ppm).

Microbial Challenge Study

A five-strain cocktail mixture of *Listeria monocytogenes* cultures was used in the study and included: *L. monocytogenes* Scott A, H7764 1/2a, H7969 4b, H7962 4b, and H7762 4b. Cultures were individually grown in trypticase soy broth containing 0.6% yeast extract (TSB-YE broth) (Difco, Becton, Dickson and Company, Sparks, Md., U.S.A.) for 24 h at 35 °C. Following incubation, 1 ml of each culture was added to 500 ml of TSB-YE broth and incubated for an additional 24 h at

35 °C. The original cocktail was diluted using sterile 0.1% peptone water (Difco, Becton Dickson and Company, Sparks, Md., U.S.A.) to target for an inoculum level of 3-log CFU/g on the frankfurters and ham slices.

Whole frankfurters and ham slices were aseptically removed from the package and surface inoculated with 1 ml of a 5-strain cocktail mixture of *L. monocytogenes*. Samples were hand massaged for 10-15 seconds to distribute microorganisms, vacuum sealed and stored at 10°C for 35 days. Evaluations of log growth were performed weekly for the first six weeks and once every two weeks for the remainder of the study. Samples were prepared in duplicate by first blending whole frankfurters with sufficient 0.1% peptone water to achieve a 1:5 dilution of each sample. Appropriate dilutions were then plated on modified oxford media (MOX) and incubated at 35°C for 48 hours to allow for enumeration of *L. monocytogenes*.

Results and Discussion

Frankfurters

Commercially available uncured, no-nitrate-or-nitrite-added frankfurters (Brands C-J) and two nitrite added controls (Brands A and B) were acquired from retail outlets and transported under refrigerated conditions to the Iowa State University Meat Laboratory (Ames, Iowa, U.S.A.). Brands were evaluated for quality attributes that are considered critical to microbial growth. Control brands A and B were widely accepted, high quality brands and were selected to demonstrate typical

L. monocytogenes inhibition. Both control brands contained the antimicrobials sodium/and or potassium lactate and/or sodium diacetate. All uncured, no-nitrate-or-nitrite added brands with the exception of Brand I, exhibited typical cured meat characteristics including color that were similar to the control. Based upon the ingredient statements (Appendix 1) Brand I was manufactured with no intention of replacing nitrite which resulted in an uncured, brownish-grey external appearance. Selected frankfurters were chosen based on the meat block formulation and were 100% all beef frankfurters to minimize variation among products as much as possible. Brand I did contain a portion of mechanically separated chicken, nevertheless, the main protein source was beef. Uncured, no-nitrate-or-nitrite-added brands E, F, and G contained sodium lactate which accounted for the significant differences ($P<0.05$) in growth of *L. monocytogenes* among uncured brands.

Sodium Chloride Analysis

Measurements for sodium chloride are reported in Figure 1. Control Brand B had a higher percentage of sodium chloride when compared to the control Brand A ($P<0.05$) but was not significantly different from uncured, no-nitrate-or-nitrite added Brands E and G. The remaining uncured, no-nitrate-or-nitrite added Brands (C, D, F, G, I and J) were not significantly different ($P>0.05$) from control Brand A. Negative correlations ($P<0.05$) were observed for sodium chloride and overall log growth throughout 35 days, indicating that a higher level of salt would result in lower maximum populations of *L. monocytogenes*. Even while sodium chloride levels in

the aforementioned products varied, they did not differ from at least one of the conventionally processed controls.

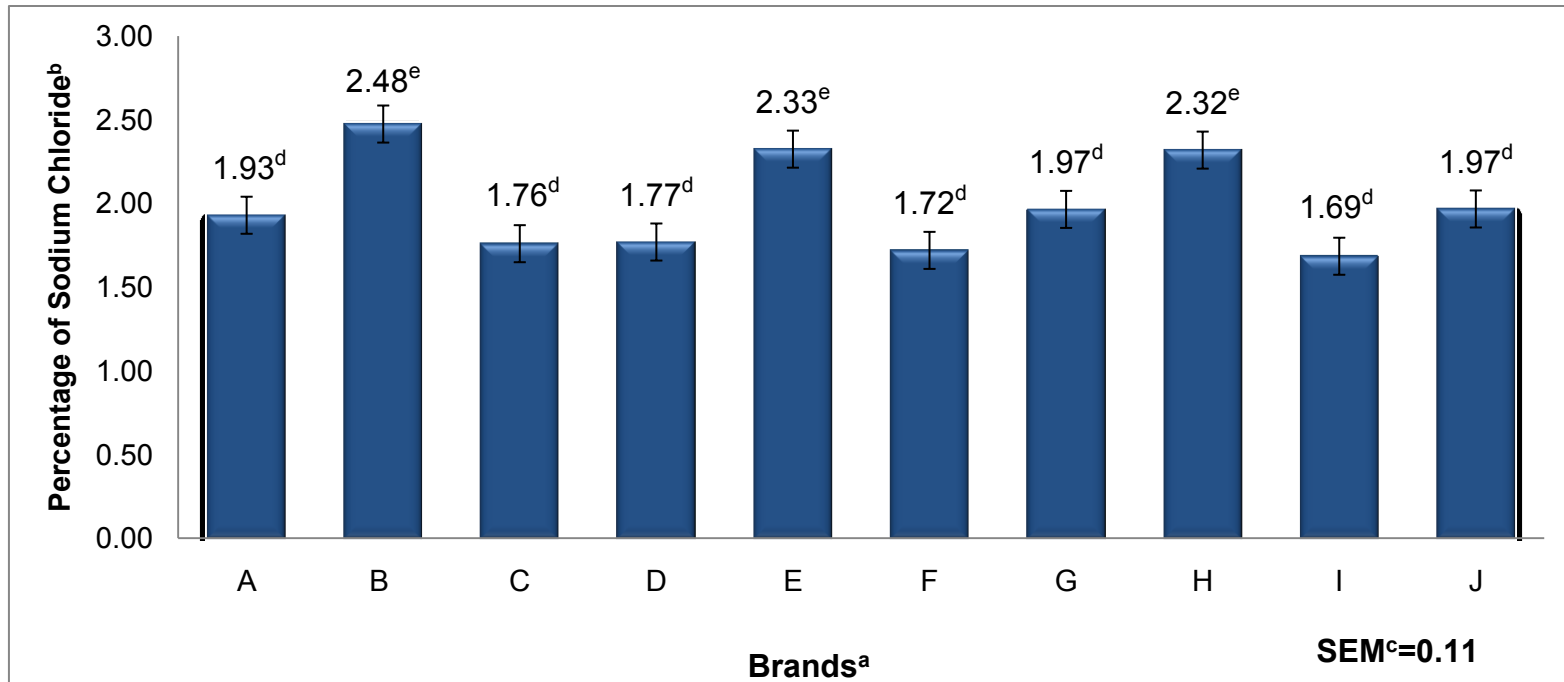
Water Activity (a_w) Measurements

No significant differences ($P>0.05$) among brands or brand by day interactions for water activity existed. Significant differences ($P<0.05$) in water activity were detected by day and are outlined in Figure 2. Despite the effects of day, there was no significant correlation ($P>0.05$) between a_w and log growth of *L. monocytogenes* over time.

pH Analysis

Brand D displayed a significantly ($P<0.05$) lower pH when compared to all other brands. Truly uncured Brand I displayed the highest pH level of all brands but was not significantly different ($P>0.05$) than all other brands including the nitrite added controls Brands A and B. In addition to the effects of day, significant brand by day interactions were also observed. No differences existed among brands on day 0 ($P>0.05$) but significant brand differences emerged by day 7 ($P<0.05$). Measurements for differences among brands and brand by day interaction for pH analysis are reported in Figure 3 and Table 1 respectively. A negative correlation ($P<0.05$) was observed for a relationship of pH to log of *L. monocytogenes* growth. This could be due to the lack of lactate present in the brands which reached their maximum population levels of *L. monocytogenes* by day 14.

Figure1. Least squares means of sodium chloride differences among uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) commercially available frankfurters.



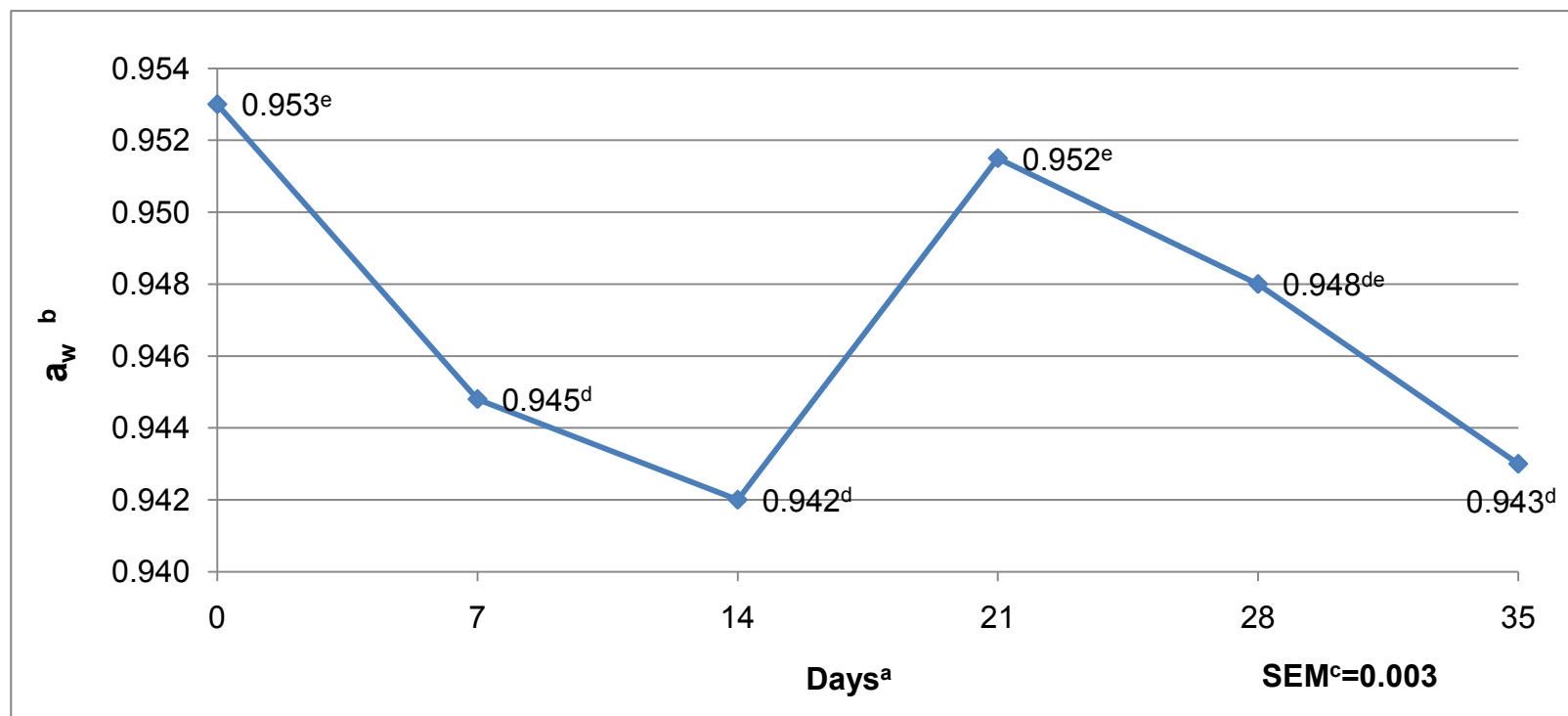
^aBrands: A-B = different brands of commercial nitrite added frankfurter product controls and C-J = uncured, no-nitrate-or-nitrite-added commercial frankfurter products.

^bSodium chloride determination reported in percentage of sample.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial frankfurter products.

^{d-e} Means with different superscripts are different ($P < 0.05$).

Figure 2. Least squares means of a_w differences on days of storage among uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) commercially available frankfurters.



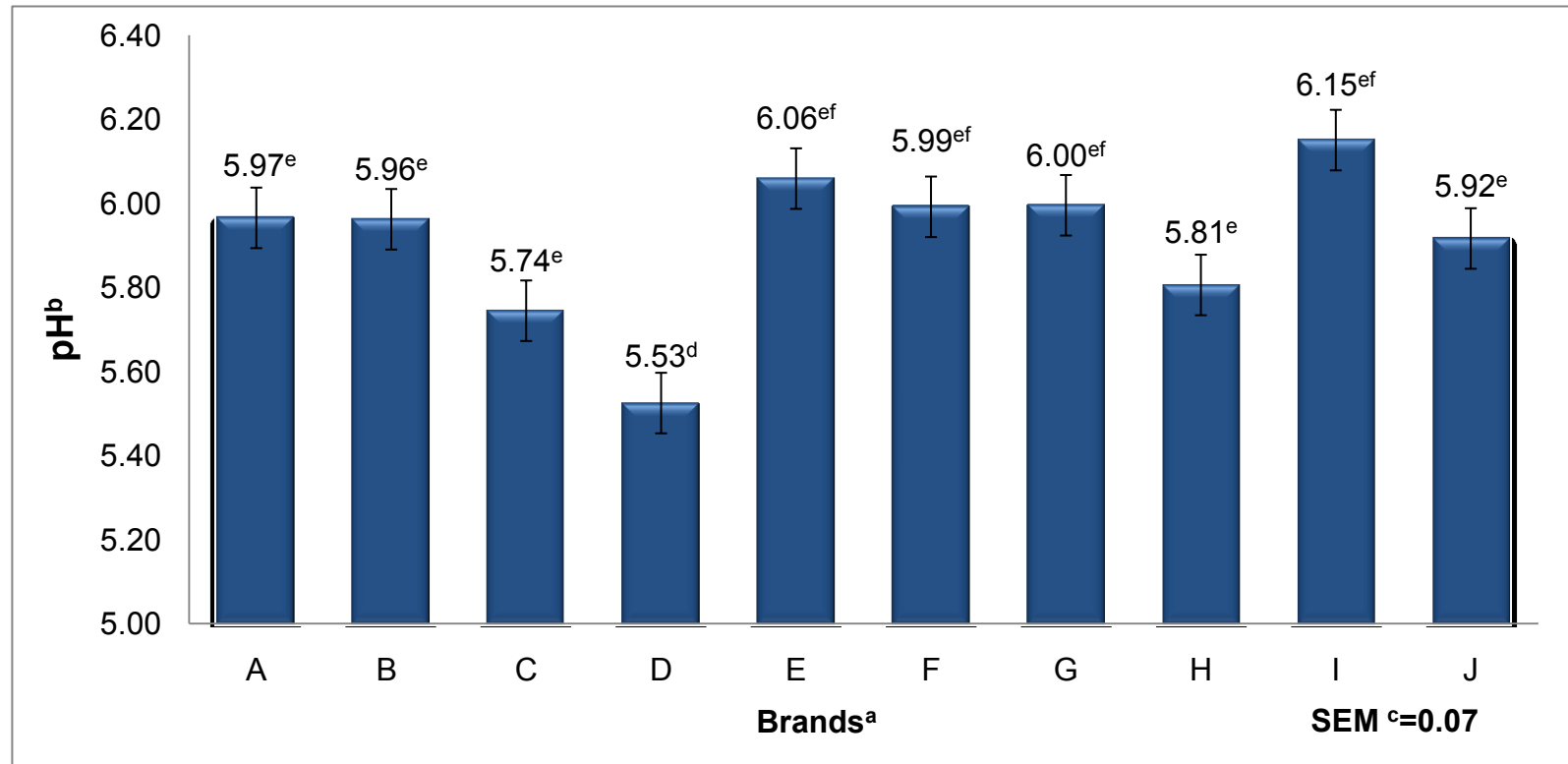
^aDays: Storage days at 10°C of all brands of commercial nitrite added frankfurter product controls and uncured, no-nitrate-or-nitrite-added commercial frankfurter products.

^bWater activity determination reported as ratio of the vapor pressure of water in a material to the vapor pressure of pure water.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial frankfurter products.

^{d-e} Means with different superscripts are different ($P < 0.05$).

Figure 3. Least squares means of pH differences among brands of uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) commercially available frankfurters.



^aBrands: A-B = different brands of commercial nitrite added frankfurter product controls and C-J = uncured, no-nitrate-or-nitrite-added commercial frankfurter products.

^bpH of commercial frankfurter products.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial frankfurter products.

^{d-f} Means with different superscripts are different (P<0.05).

Table 1. Least squares means for the interaction brands with storage time for pH^k of uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) commercially available frankfurters.

Brand ^b	Day ^a					
	0	7	14	21	28	35
A	5.98 ^d	6.02 ^{dg}	5.96 ^e	5.96 ^{eh}	5.93 ^f	5.95 ^f
B	6.01 ^d	5.99 ^{df}	5.97 ^e	5.95 ^{ef}	5.95 ^f	5.92 ^f
C	6.08 ^d	6.17 ^{efghij}	5.83 ^e	5.76 ^e	5.44 ^{de}	5.19 ^d
D	5.99 ^d	5.84 ^d	5.47 ^d	5.32 ^d	5.23 ^d	5.17 ^d
E	6.08 ^d	6.23 ^{efghij}	6.14 ^{ei}	6.12 ^{fghi}	5.90 ^f	5.89 ^f
F	6.01 ^d	6.04 ^{di}	6.07 ^{eh}	6.00 ^{ej}	5.86 ^f	5.96 ^f
G	6.00 ^d	6.07 ^{dj}	5.99 ^{eg}	5.98 ^{ei}	5.97 ^f	5.97 ^f
H	6.15 ^d	6.03 ^{dh}	5.96 ^e	5.71 ^e	5.53 ^f	5.46 ^{de}
I	6.19 ^d	6.30 ^{fghij}	6.28 ^{fghi}	6.28 ^{ij}	6.03 ^f	5.83 ^f
J	6.12 ^d	5.98 ^{de}	5.98 ^{ef}	5.95 ^{eg}	5.82 ^f	5.63 ^{ef}
SEM^c	0.04	0.04	0.04	0.04	0.04	0.04

^aDays of storage over 35 day study for commercial frankfurter products

^bBrands: A-B = different brands of commercial nitrite added frankfurter product controls and C-J = uncured, no-nitrate-or-nitrite-added commercial frankfurter products.

^cSEM = Pooled standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial frankfurter products.

^{d-j} Means within column with different superscripts are different (P<0.05).

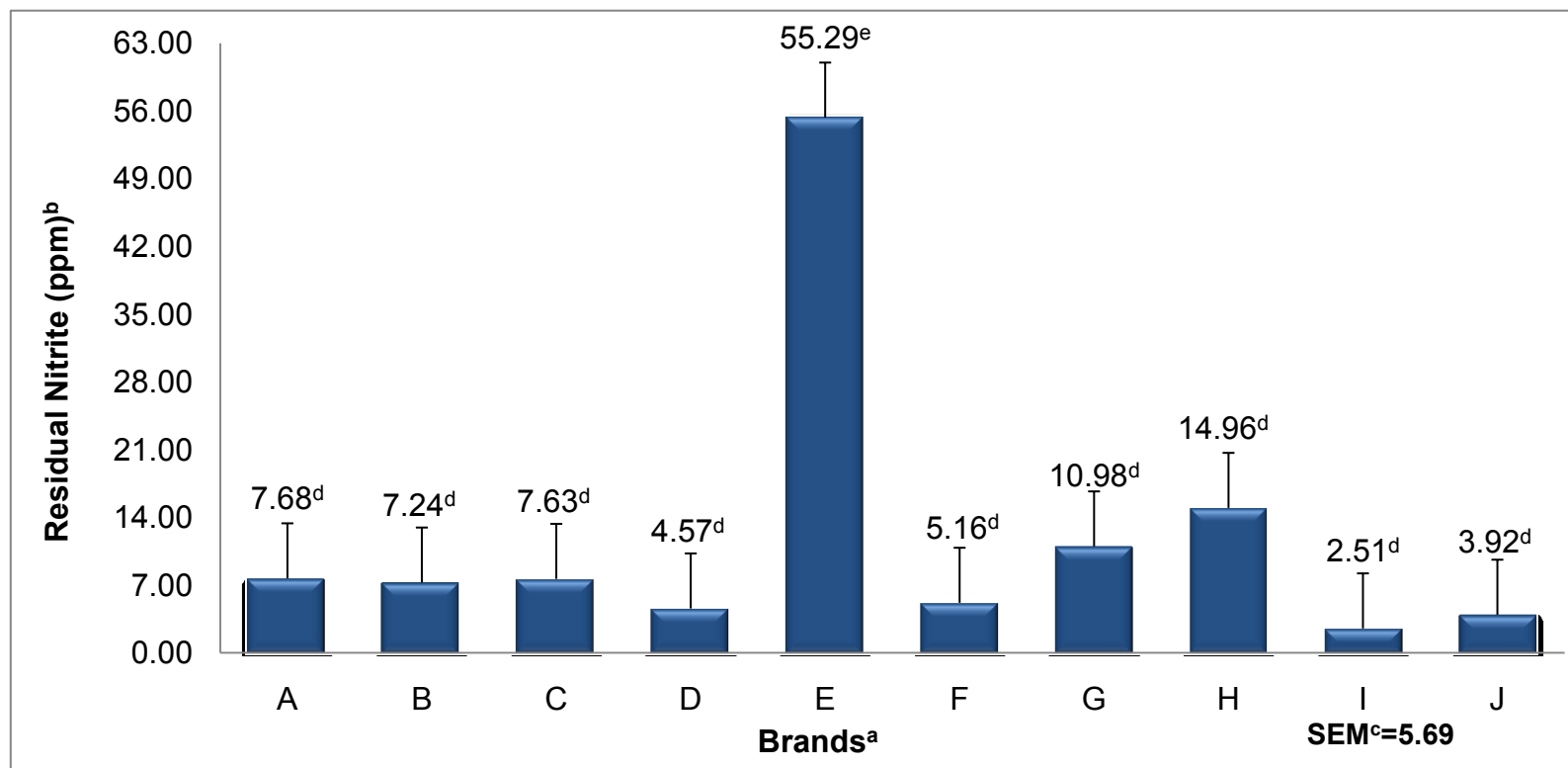
^kpH of commercial frankfurter products.

Residual Nitrate and Nitrite Analysis

Truly uncured Brand I exhibited a significantly ($P < 0.05$) lower level of residual nitrite than Brand D, but was not significantly different ($P > 0.05$) from the nitrite added controls and remaining uncured, no-nitrate-or-nitrite added frankfurter brands (Figure 4). It has been reported that residual nitrite levels decrease throughout the storage period (Cassens 1997b) and the depletion rate is influenced by storage temperature (Hustad and others 1973). As a result of direct nitrite addition during the production process we would expect the nitrite added controls, as well as a portion of the uncured, no-nitrate-or-nitrite-added brands, to possess a higher level of residual nitrite than the truly uncured Brand I. Nevertheless, this may be an indication that the nitrite added controls and uncured, no-nitrate-or-nitrite-added brands which were not significantly different ($P > 0.05$) may have been approaching the end of their shelf life or were temperature abused during distribution or display in retail establishments. While significant differences existed among brands, there was no significant correlation ($P > 0.05$) of residual nitrite to log growth of *L. monocytogenes*.

Residual nitrate levels of uncured, no-nitrate-or-nitrite-added frankfurters are displayed in Figure 5. As with residual nitrite, truly uncured Brand I possessed the lowest residual nitrate concentration ($P < 0.05$) among all other brands. Uncured no-nitrate-or-nitrite-added Brands E and H had the highest level of residual nitrate

Figure 4. Least squares means of residual nitrite differences among brands of uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) commercially available frankfurters.



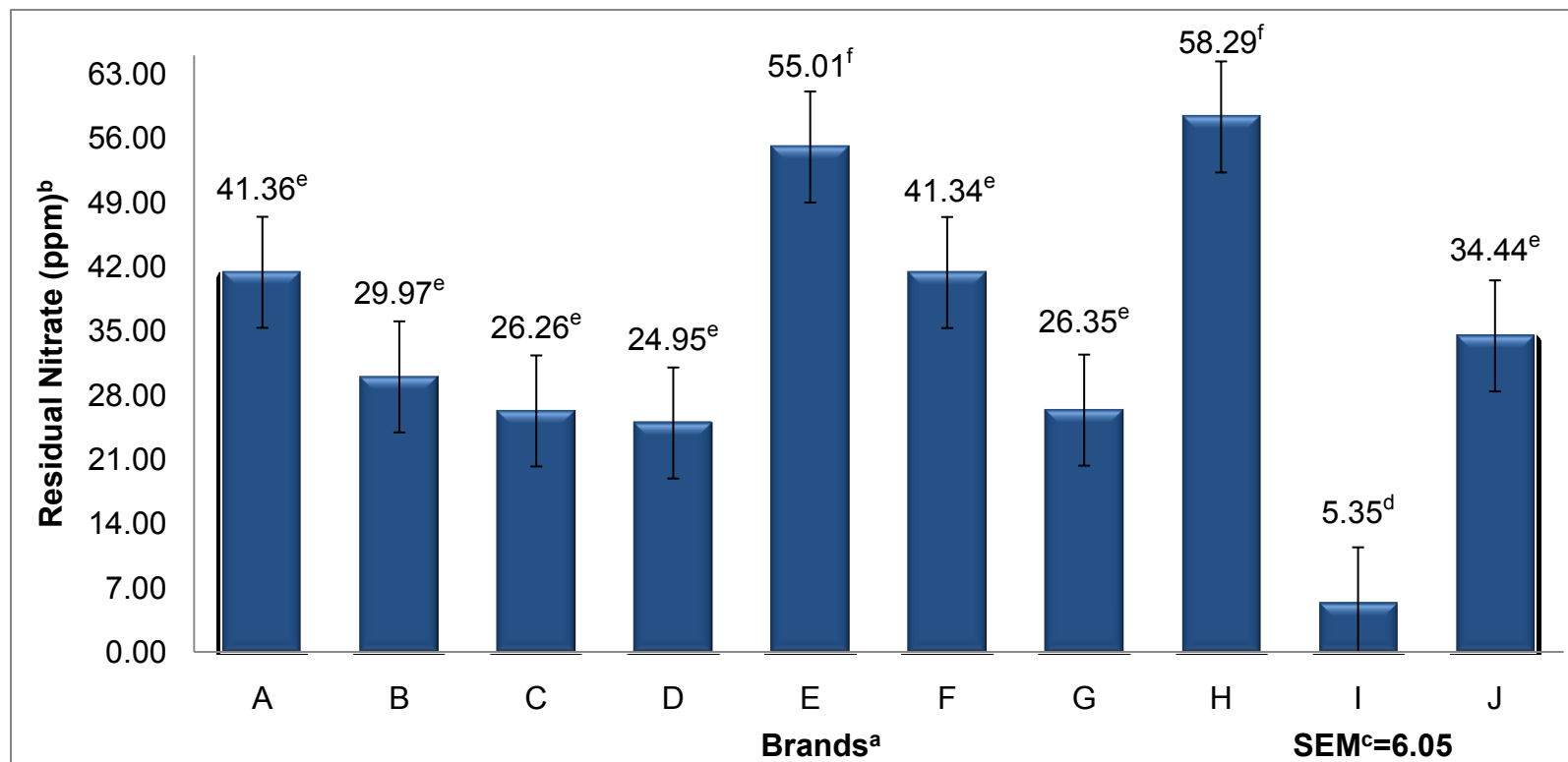
^aBrands: A-B = different brands of commercial nitrite added frankfurter product controls and C-J = uncured, no-nitrate-or-nitrite-added commercial frankfurter products.

^bResidual nitrite determination reported in ppm of sample.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial frankfurter products.

^{d-e} Means with different superscripts are different (P<0.05).

Figure 5. Least squares means of residual nitrate differences among brands of uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) commercially available frankfurters.



^aBrands: A-B = different brands of commercial nitrite added frankfurter product controls and C-J = uncured, no-nitrate-or-nitrite-added commercial frankfurter products.

^bResidual nitrate determination reported in ppm of sample.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial frankfurter products.

^{d-f} Means with different superscripts are different ($P < 0.05$).

($P < 0.05$) among all other brands including the nitrite added controls, but were not different from each other ($P > 0.05$). In addition, a significant brand by day interaction was present for residual nitrate and those least squares means are reported in Table 2. Residual nitrate was not correlated ($P > 0.05$) and thus is not strongly related to the log growth of *L. monocytogenes*.

Growth of Listeria monocytogenes

Figure 6 shows the growth of all ten commercial brands over the sampling period. Control Brands A and B, containing sodium nitrite, lactate, and diacetate, resulted in little or no growth throughout the accelerated challenge study. Furthermore, it is clear that the no-nitrate-or-nitrite-added Brands C-J were unable to repress the growth of *L. monocytogenes* throughout the 35 day sampling period. No-nitrate-or-nitrite-added brands exhibited a decreased lag time and shorter generation time for initiation of growth, resulting in a greater population of *L. monocytogenes* when compared to the sodium nitrite, sodium lactate/diacetate controls ($P < 0.05$). Rate of growth over the first 14 days was greatest ($P < 0.05$) for uncured Brands I (truly uncured) and C (alternative cure without lactate) over all other brands except H (alternative cure without lactate). Brand H had a higher growth rate ($P < 0.05$) than uncured, no-nitrate-or-nitrite-added Brands F and D as well as the nitrite added controls (Brands A and B). Brands that contained lactate had growth rates that were similar ($P > 0.05$) to both controls (A, B).

Table 2. Least squares means for the interaction brands with storage time for residual nitrate^c of uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) commercially available frankfurters.

Brand ^b	Day ^a		
	0	14	35
A	39.91 ^{fe}	40.58 ^{fi}	43.60 ^{ghijk}
B	30.04 ^f	29.28 ^{fj}	30.60 ^{fi}
C	29.47 ^f	25.38 ^{fg}	23.93 ^f
D	24.90 ^f	23.31 ^{ef}	26.63 ^{fg}
E	53.75 ^{ghi}	57.71 ⁱ	54.35 ^k
F	39.19 ^{fh}	43.40 ^{ghij}	41.42 ^{fk}
G	23.65 ^f	26.75 ^f	28.65 ^f
H	72.77 ^j	74.98 ^k	27.13 ^{fh}
I	6.20 ^e	5.15 ^e	4.71 ^e
J	35.37 ^{fg}	32.89 ^{fh}	35.06 ^{fj}
SEM^d	6.27	6.27	6.27

^aDays of storage over 35 day study for commercial frankfurter products

^bBrands: A-B = different brands of commercial nitrite added frankfurter product controls and C-J = uncured, no-nitrate-or-nitrite-added commercial frankfurter products.

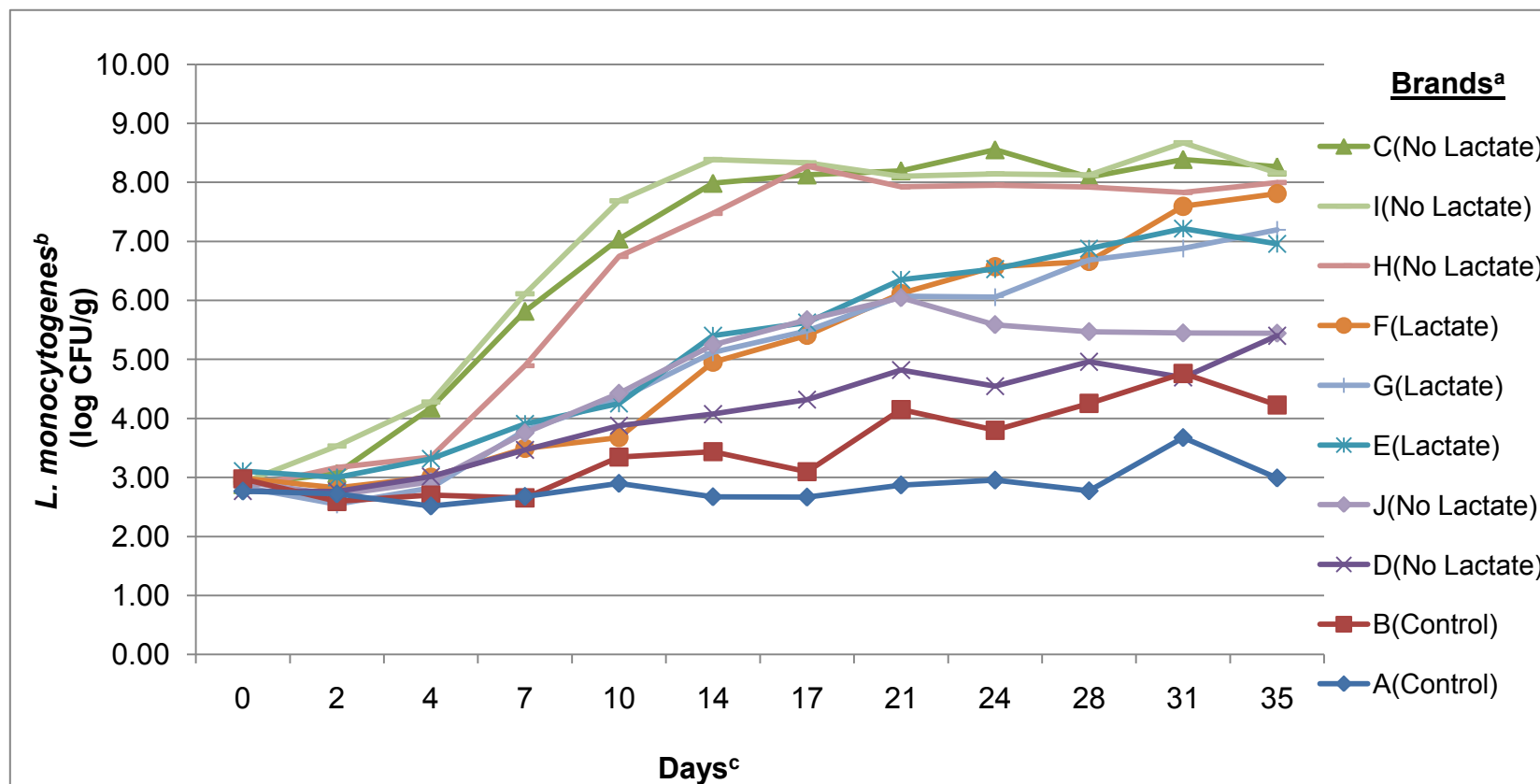
^cResidual nitrate determination reported in ppm.

^dSEM = Pooled standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial frankfurter products.

^{e-k} Means within column with different superscripts are different (P<0.05).

Differences among brands of uncured, no-nitrate-or-nitrite-added frankfurters were observed and group contrasts were performed to determine how uncured, no-nitrate-or-nitrite-added products which contained lactate were different in growth when compared to either uncured, no-nitrate-or-nitrite-added products without lactate added or the conventionally cured controls. Results of the group contrast are outlined in Figure 7 and Table 3. No significant differences ($P>0.05$) were observed between the conventionally cured controls and uncured brands containing lactate, and the uncured brands containing lactate versus those uncured brands which did not contain lactate on day 0, however significant differences ($P<0.05$) were observed by day 7. Significant differences remained throughout the 35 day challenge study for conventionally cured brands versus uncured brands without lactate and conventionally cured brands versus uncured brands with lactate ($P<0.05$). On day 21, however, no significant differences ($P>0.05$) were observed between the overall growth of uncured brands containing lactate versus those uncured, no-nitrate-or-nitrite-added brands which did contain lactate. While the addition of lactate at reduced levels provides some suppression of growth in uncured, no-nitrate-or-nitrite added products, it is not equal to that of our conventionally cured frankfurters which contain direct nitrite and lactate and diacetate. Correlation values between log growth of *L. monocytogenes* and salt, pH, residual nitrite and water activity (a_w) are reported in Table 4.

Figure 6. Growth of *Listeria monocytogenes* among brands of uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) commercially available frankfurters.

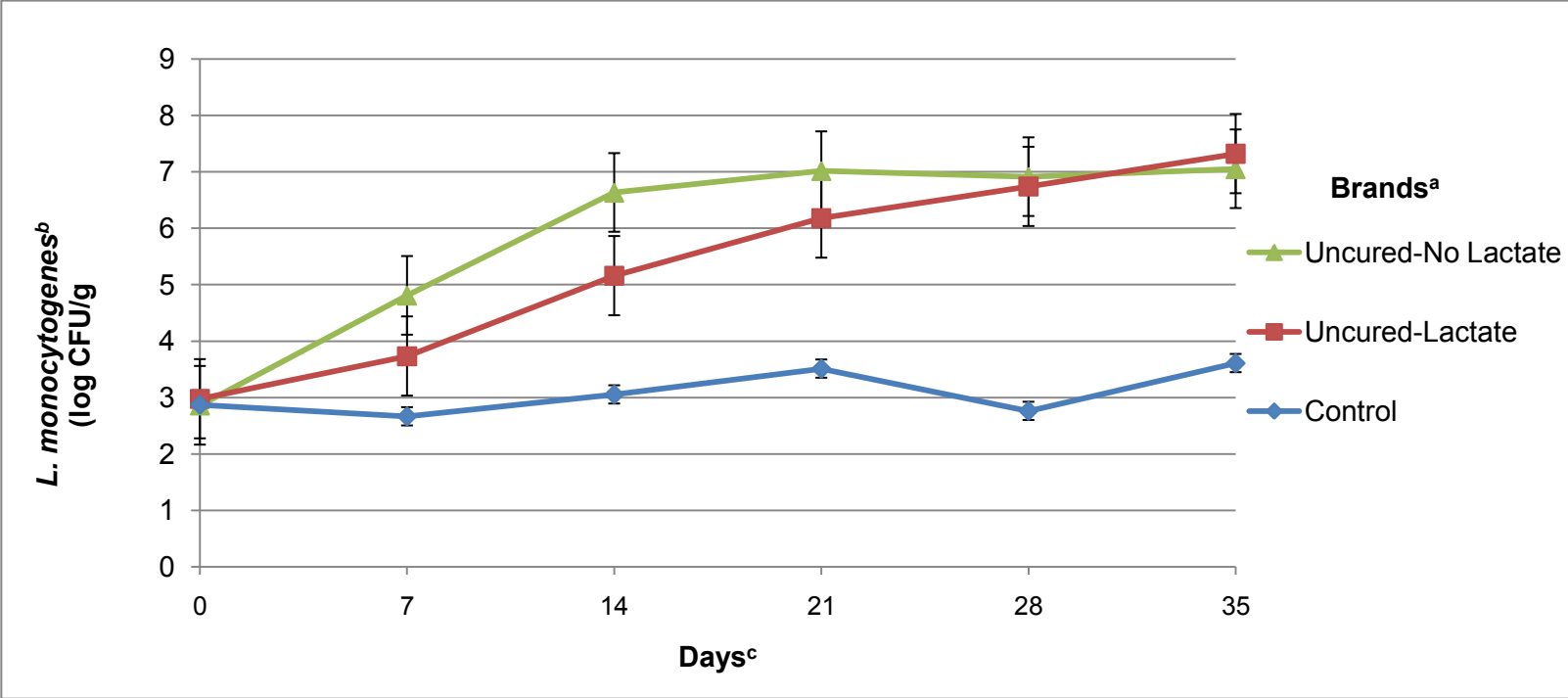


^aBrands: A-B = different brands of commercial nitrite added frankfurter product controls and C-J = uncured, no-nitrate-or-nitrite-added commercial frankfurter products.

^bGrowth of *L.monocytogenes* reported in log CFU/g.

^cDays of storage at 10°C over a 35 day study for commercial frankfurter products.

Figure 7. Growth of *Listeria monocytogenes* among brands of uncured, no-nitrate-or-nitrite-added (Brand C-J) containing lactate (Brand E-G) or no lactate (Brand C-D and H-J) and nitrite added (Brand A-B) commercially available frankfurters.



^aBrands: C-J = uncured, no-nitrate-or-nitrite-added brands with Brand E-G uncured with lactate and Brand C-D and H-J uncured no lactate and nitrite added (Brand A-B) commercially available frankfurters.

^bGrowth of *L.monocytogenes* reported in log CFU/g.

^cDays of storage at 10°C over a 35 day study for commercial frankfurter products.

Table 3. P-values for comparison of growth of *L. monocytogenes* among brands of uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) commercially available frankfurters.

Brand ^a Comparison	Day ^b					
	0	7	14	21	28	35
Nitrite Control - Uncured No Lactate	0.885	0.0003**	0.0004**	0.0016**	0.0006**	0.0004**
Nitrite Control - Uncured with Lactate	0.2316	0.0280**	0.0164**	0.0127**	0.0015**	0.0005**
Uncured with Lactate- Uncured No Lactate	0.1010*	0.0093**	0.0298**	0.2539	0.7716	0.6425

^aBrands: C-J = uncured, no-nitrate-or-nitrite-added brands. Brand E-G uncured with lactate and Brand C-D and H-J uncured no lactate and nitrite added (Brand A-B) commercially available frankfurters.

^bDays of storage at 10°C over a 35 day study for commercial frankfurter products.

*(P<0.10) trend for groups to be different.

** (P<0.05) groups are significantly different.

Table 4. Pearson's correlation values^a of quality attributes to log growth of *L. monocytogenes* on uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) commercially available frankfurter products.

<i>Salt</i>	<i>Nitrite(ppm)</i>	<i>pH</i>	<i>a_w</i>
-0.1912**	0.07181	-0.25806**	0.02835

^aR-Values reported

** (P<0.05) groups are significantly correlated

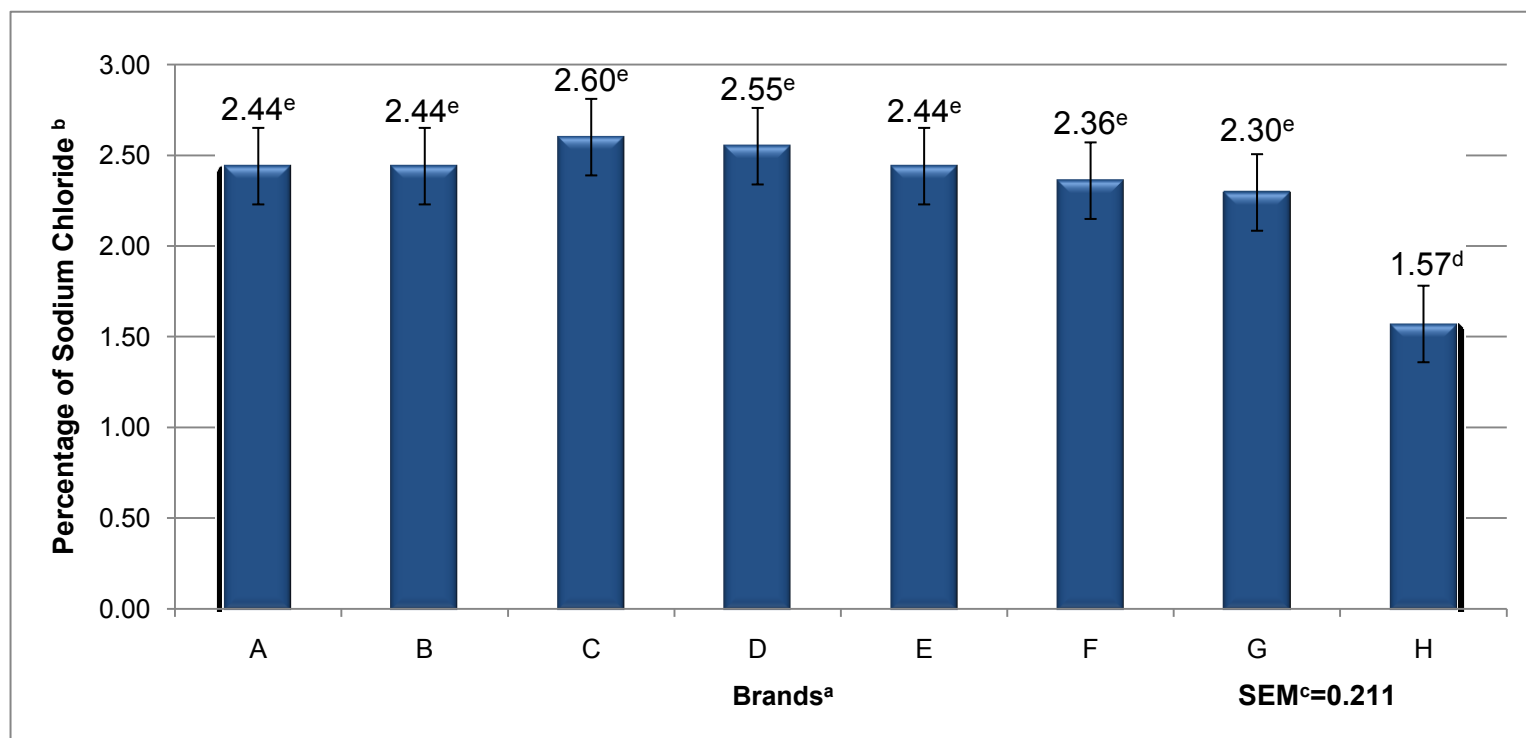
Deli Ham

Commercially available uncured, no-nitrate-or-nitrite-added deli ham products (Brands D-H) and three nitrite added controls (Brands A, B and C) were acquired from retail outlets and transported under refrigerated conditions to the Iowa State University Meat Laboratory (Ames, Iowa, U.S.A.). Brands were evaluated for quality attributes that are considered critical to microbial growth. Control brands A, B and C were all widely accepted, high quality brands and were selected to demonstrate typical *L. monocytogenes* inhibition. All control brands contained the antimicrobials sodium/and or potassium lactate and sodium diacetate. An increased number of nitrite added controls were used for this portion of the study as preliminary challenge studies (data not shown) showed no significant differences between a conventionally processed control and uncured, no-nitrate-or-nitrite-added brands. All uncured, no-nitrate-or-nitrite-added brands exhibited typical cured meat characteristics including color that were similar to the control and, based upon the ingredient statements (Appendix 2), all were manufactured with the intention of replacing nitrite. Uncured, no-nitrate-or-nitrite-added Brands E and H contained sodium lactate in the ingredient statement, however, there were no significant differences in log growth accounted for between uncured, no-nitrate-or-nitrite-added deli hams.

Sodium Chloride Analysis

Measurements for sodium chloride are reported in Figure 8. Uncured, no-nitrate-or-nitrite-added Brand H had the lowest percentage of sodium chloride when compared to all other brands including the nitrite added controls ($P < 0.05$). No

Figure 8. Least squares means of sodium chloride differences among uncured, no-nitrate-or-nitrite-added (Brand D-H) and nitrite added (Brand A-C) commercially available deli hams.



^aBrands: A-C = different brands of commercial nitrite added deli ham product controls and D-H = uncured, no-nitrate-or-nitrite-added commercial deli ham products.

^bSodium chloride determination reported in percentage of sample.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial deli ham products.

^{d-e} Means with different superscripts are different (P<0.05).

correlations ($P>0.05$) were observed for sodium chloride and overall log growth throughout 35 days, indicating that sodium chloride levels did cause a significant difference in growth of *L. monocytogenes* between uncured and conventional brands.

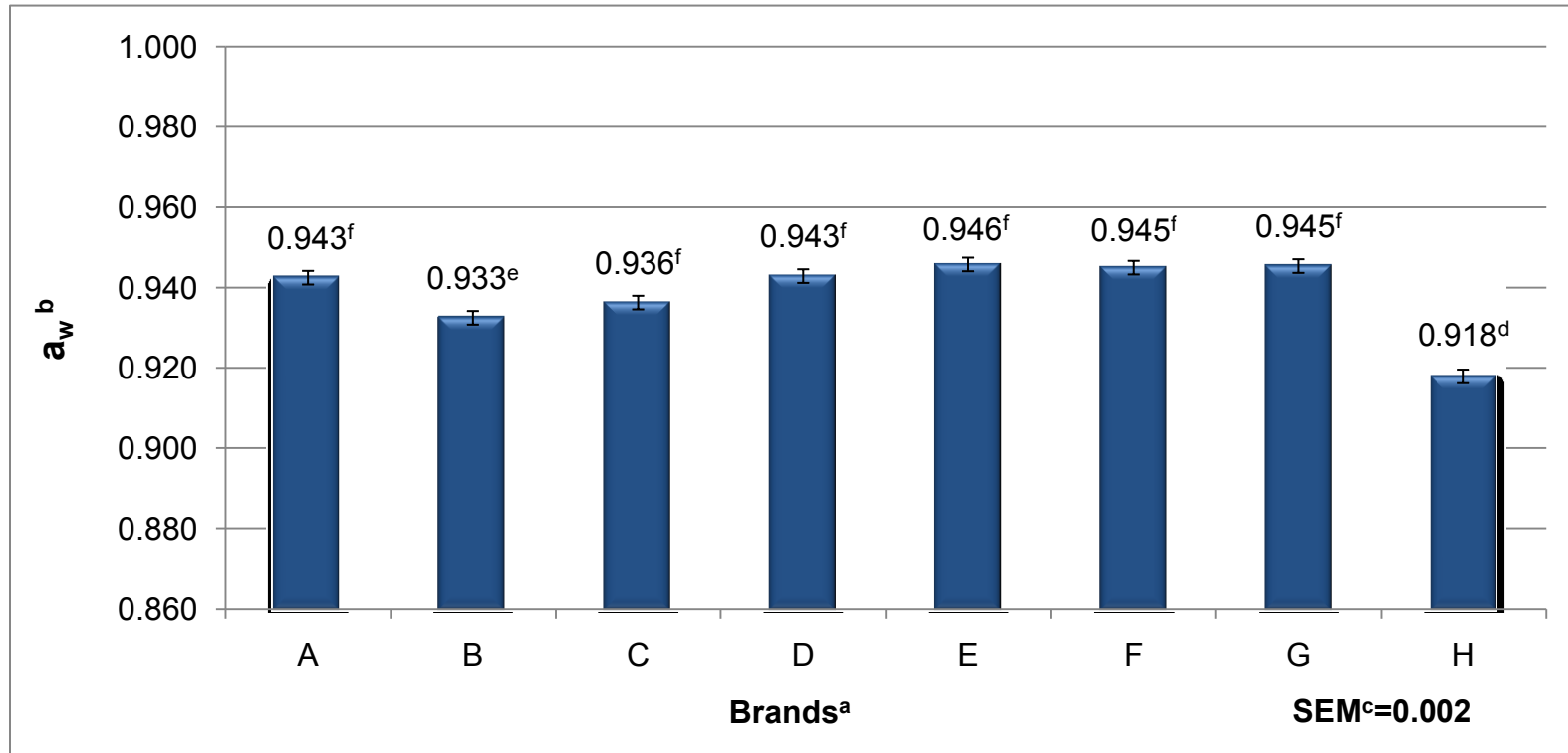
Water Activity (a_w) Measurements

Significant differences ($P<0.05$) in water activity were detected by brand and are outlined in Figure 9. Control Brands B and C were significantly lower ($P<0.05$) in water activity among all other brands including control Brand A however they were not significantly different from each ($P>0.05$). All uncured, no-nitrate-or-nitrite-added Brands D-H were not significantly different from the Brand A control ($P>0.05$). A positive correlation was observed ($P<0.05$) between a_w and log growth of *L. monocytogenes* over time, which would be expected as a higher moisture level would result in faster growth for *L. monocytogenes*.

pH Analysis

Measurements for pH analysis established a significant difference among brands as outlined in Figure 10. Uncured, no-nitrate-or-nitrite-added Brands D, E, F, G, and H were not significantly ($P>0.05$) different from at least one of the nitrite added controls (Brands A-C). In addition to differences among brands, a significant ($P<0.05$) day by brand interaction was present for pH and those least squares means are reported in Table 5. A negative correlation ($P<0.05$) was also observed for a relationship of pH to log of *L. monocytogenes* growth.

Figure 9. Least squares means of a_w differences among uncured, no-nitrate-or-nitrite-added (Brand D-H) and nitrite added (Brand A-C) commercially available deli hams.



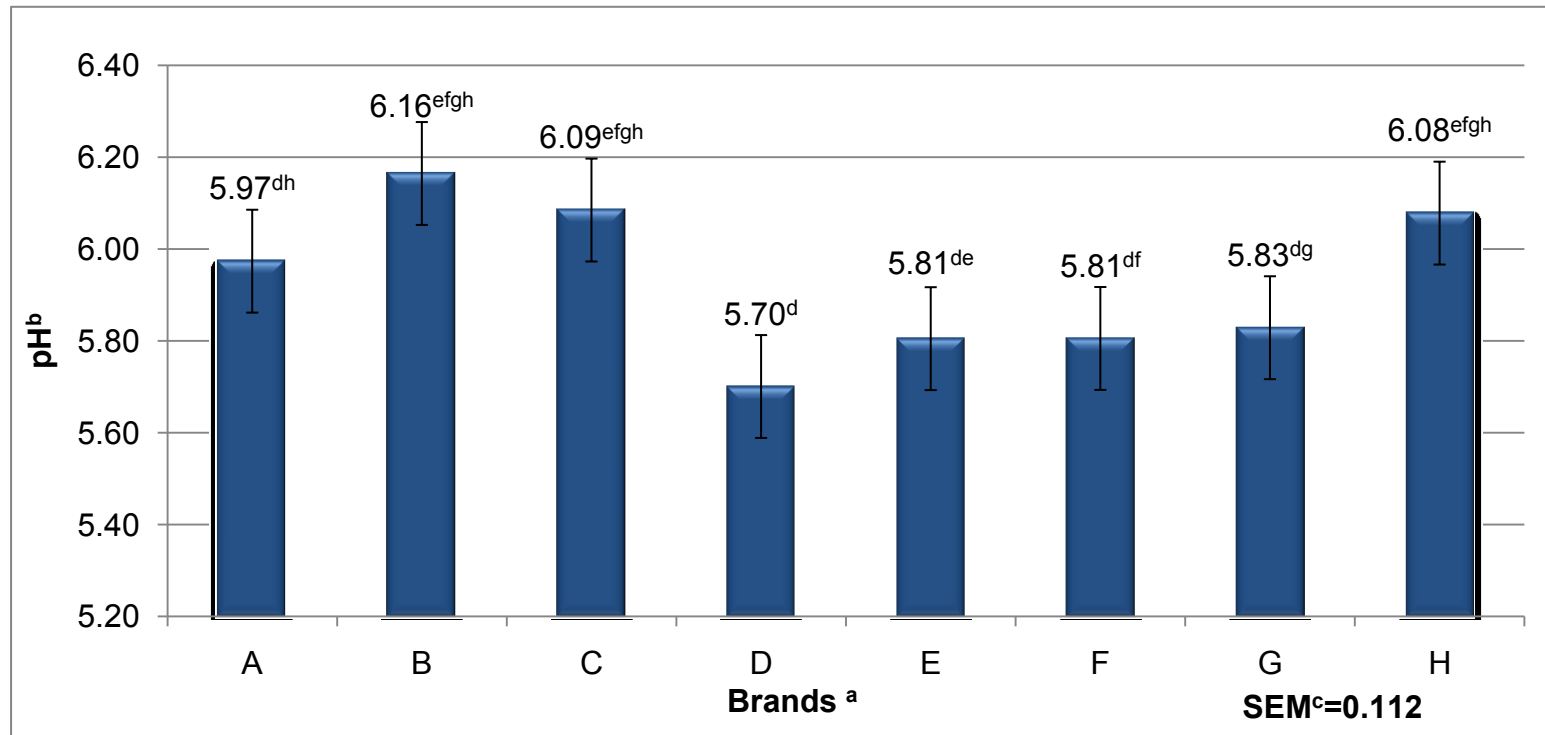
^aBrands: A-C = different brands of commercial nitrite added deli ham product controls and D-H = uncured, no-nitrate-or-nitrite-added commercial deli ham products.

^bWater activity determination reported as ratio of the vapor pressure of water in a material to the vapor pressure of pure water.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial frankfurter products.

^{d-f} Means with different superscripts are different ($P < 0.05$).

Figure 10. Least squares means of pH differences among uncured, no-nitrate-or-nitrite-added (Brand D-H) and nitrite added (Brand A-C) commercially available deli hams.



^aBrands: A-C = different brands of commercial nitrite added deli ham product controls and D-H = uncured, no-nitrate-or-nitrite-added commercial deli ham products.

^bpH of commercial ham products.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial frankfurter products.

^{d-h} Means with different superscripts are different (P<0.05).

Table 5. Least squares means for the interaction brands with storage time for pH^a of uncured, no-nitrate-or-nitrite-added (Brand D-H) and nitrite added (Brand A-C) commercially available deli ham products.

Brand ^c	Day ^b					
	0	7	14	21	28	35
A	6.09 ^d	6.20 ^d	5.87 ^{dh}	5.96 ^d	5.85 ^{df}	5.87 ^{efi}
B	6.17 ^d	6.20 ^d	6.15 ^{fgh}	6.17 ^d	6.14 ^{fgh}	6.16 ^{hi}
C	6.07 ^d	6.06 ^d	6.04 ^{efgh}	6.09 ^d	6.09 ^{fghi}	6.16 ^{hi}
D	6.05 ^d	5.82 ^d	5.54 ^d	6.16 ^d	5.43 ^d	5.21 ^{dg}
E	6.08 ^d	5.88 ^d	5.68 ^{de}	5.79 ^d	5.76 ^{dgi}	5.64 ^{efg}
F	6.02 ^d	6.06 ^d	5.74 ^{df}	5.78 ^d	5.65 ^{de}	5.59 ^{df}
G	6.06 ^d	6.05 ^d	5.86 ^{dg}	5.76 ^d	5.71 ^{df}	5.52 ^{de}
H	6.20 ^d	6.24 ^d	6.14 ^{fgh}	6.16 ^d	5.90 ^{efgh}	5.83 ^{efg}
SEM^d	0.15	0.15	0.15	0.15	0.15	0.15

^apH of commercial deli ham products.

^bDays of storage over a 35 day study for commercial deli ham products

^cBrands: A-C = different brands of commercial nitrite added frankfurter product controls and D-H= uncured, no-nitrate-or-nitrite-added commercial deli ham products.

^dSEM = Pooled standard error standard of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial deli ham products.

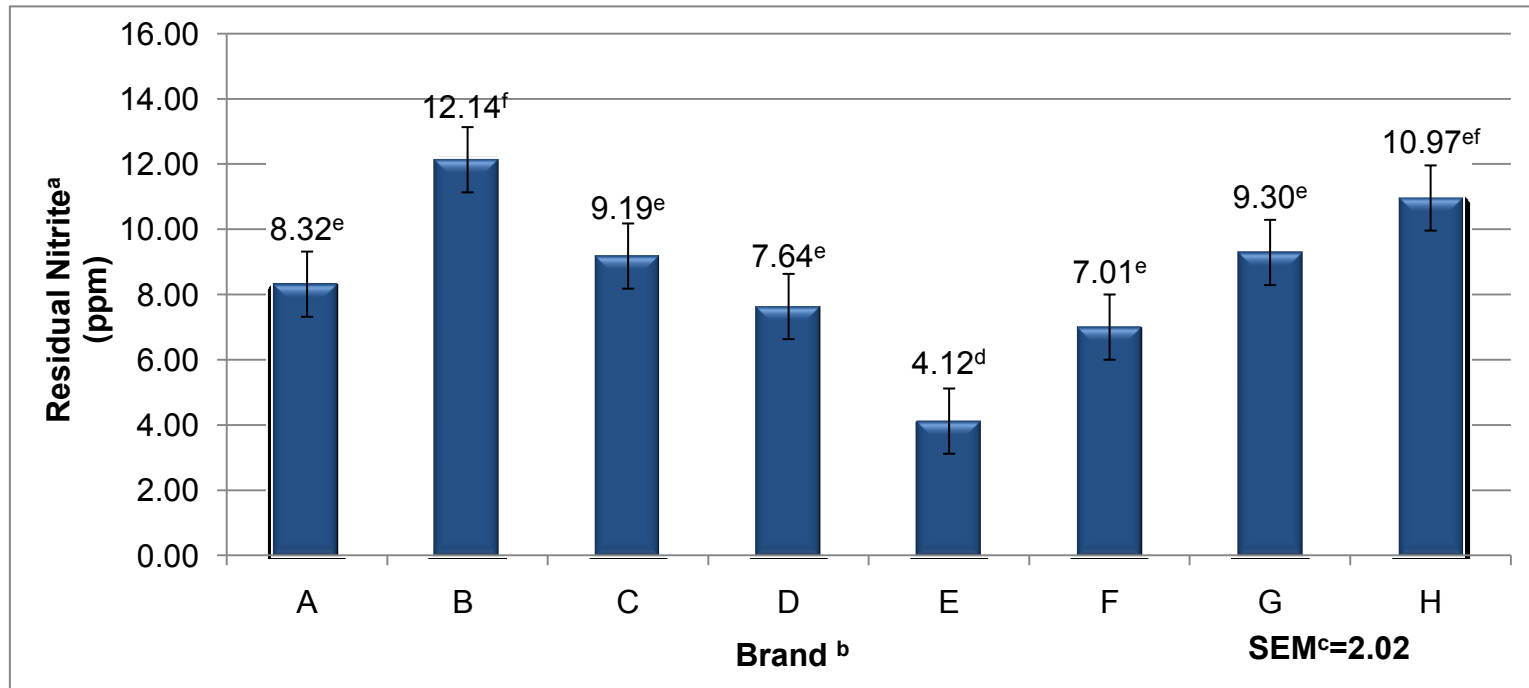
^{d-i} Means within column with different superscripts are different (P<0.05).

Residual Nitrate and Nitrite Analysis

The least square means for residual nitrite differences among brands is reported in Figure 11. Residual nitrite analysis revealed that uncured, no-nitrate-or-nitrite-added Brand E was significantly ($P < 0.05$) lower than all other brands of ham. There were no significant differences between the nitrite added controls ($P > 0.05$) and the uncured, no-nitrate-or-nitrite-added Brands D, F, G, and H were not significantly different from each other or any of the nitrite added controls ($P > 0.05$). There were no significant brand by day interactions however, there was a trend for correlation ($P < 0.10$) of residual nitrite to log growth of *L. monocytogenes*.

A wide variation existed for residual nitrate levels among the nitrite added controls as well as the uncured, no-nitrate-or-nitrite-added brands. The least squared means are displayed in Figure 12. As with residual nitrite, uncured, no-nitrate-or-nitrite-added Brand E possessed the lowest residual nitrate concentration ($P < 0.05$) however, it was not significantly different ($P > 0.05$) from the Brand A (nitrite added control) and uncured, no-nitrate-or-nitrite-added Brands E, G, and H. Uncured no-nitrate-or-nitrite-added Brands E and H had the highest level of residual nitrate ($P < 0.05$) among all other brands including the nitrite added controls, but were not different from each other ($P > 0.05$). In addition, no significant brand by day interaction was present for residual nitrate. Residual nitrate was negatively correlated ($P < 0.05$) to the log growth of *L. monocytogenes*, indicating a higher level of residual nitrate resulted in less growth over the 35 day challenge study.

Figure 11. Least squares means of residual nitrite^a differences among uncured, no-nitrate-or-nitrite-added (Brand D-H) and nitrite added (Brand A-C) commercially available deli hams.



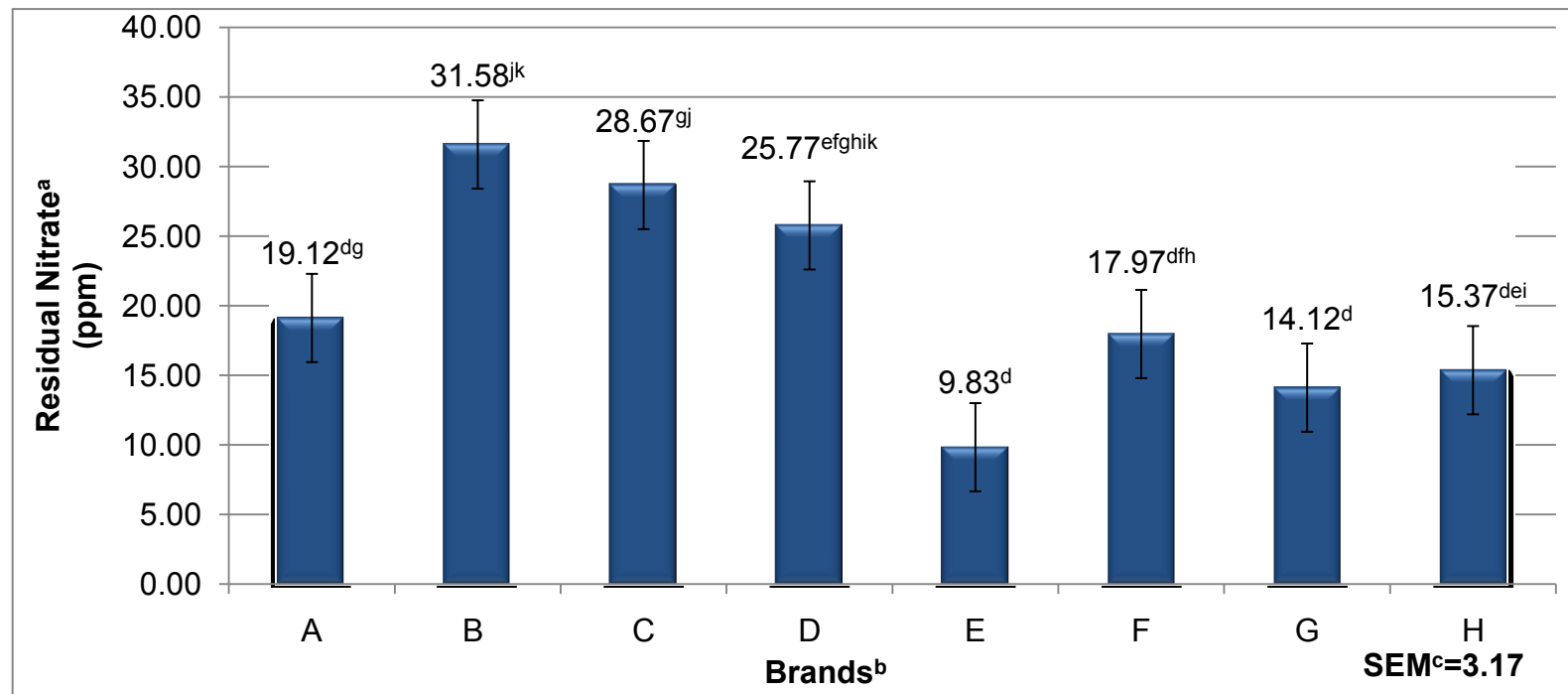
^aResidual nitrite determination reported in ppm

^bBrands: A-C = different brands of commercial nitrite added deli ham product controls and D-H = uncured, no-nitrate-or-nitrite-added commercial deli ham products.

^cSEM = Pooled standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial deli ham products.

^{d-f}Means with different superscripts are different (P<0.05).

Figure 12. Least squares means of residual nitrate^a differences among uncured, no-nitrate-or-nitrite-added (Brand D-H) and nitrite added (Brand A-C) commercially available deli hams.



^aResidual nitrate determination reported in ppm

^bBrands: A-C = different brands of commercial nitrite added deli ham product controls and D-H = uncured, no-nitrate-or-nitrite-added commercial deli ham products.

^cSEM = Pooled standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial deli ham products.

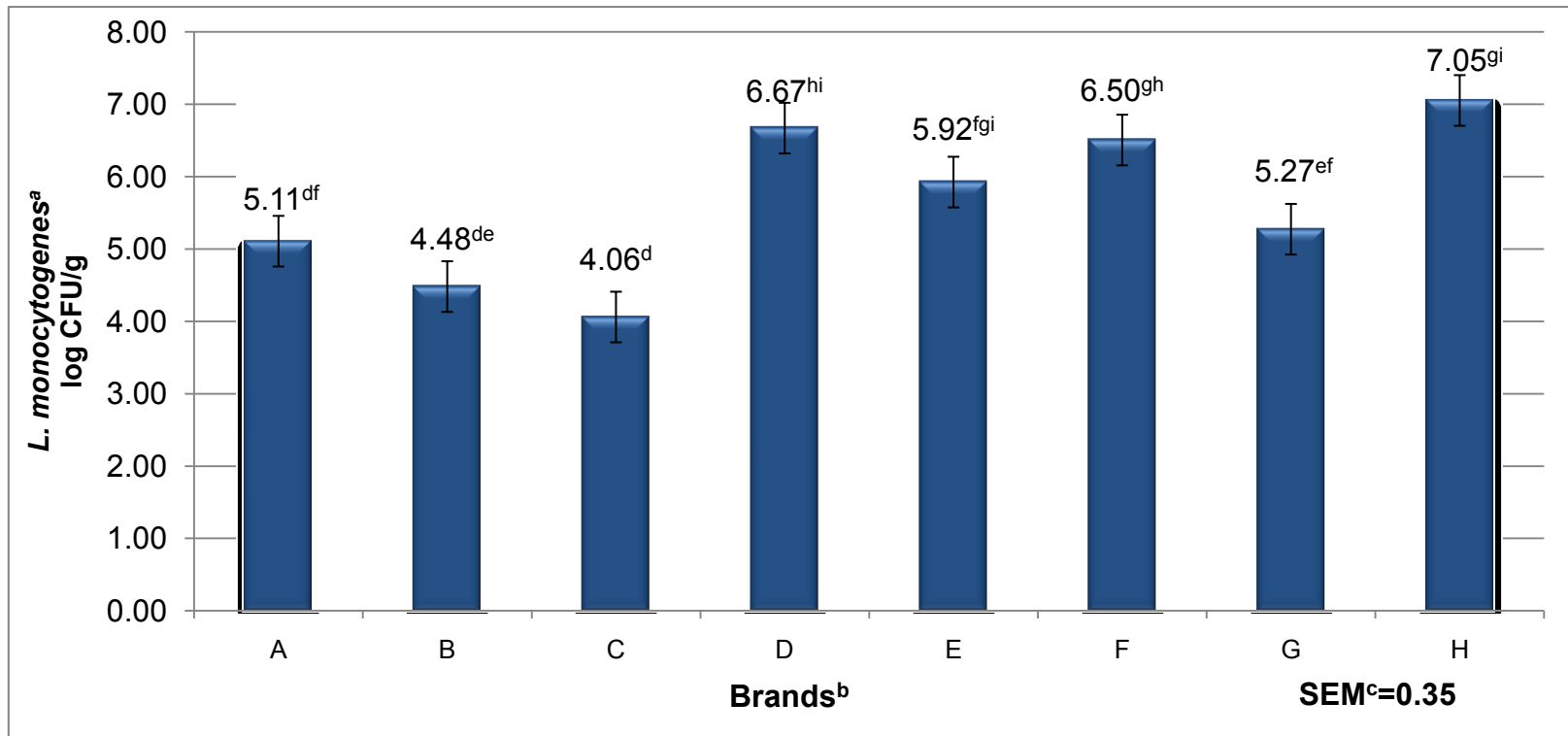
^{d-k}Means with different superscripts are different ($P < 0.05$).

Growth of Listeria monocytogenes

Figure 13 displays the least squares means of growth of *L. monocytogenes* among all eight commercial brands of deli ham over the sampling period. Control Brand C resulted in little or no growth throughout the accelerated challenge study and while exhibited the least amount of growth it was not significantly different ($P>0.05$) from nitrite added controls Brands A and B. No-nitrate-or-nitrite-added Brands E and G were not significantly different from Brand A control, however, Brands D, F, and H resulted in a greater population of *L. monocytogenes* when compared to all (Brands A-C) sodium nitrite, sodium lactate/diacetate controls ($P<0.05$).

Differences among groups of uncured, no-nitrate-or-nitrite-added hams were observed and group contrasts were performed to determine how uncured, no-nitrate-or-nitrite-added products which contained lactate were different in growth when compared to either uncured, no-nitrate-or-nitrite-added products without lactate added or the conventionally cured controls. Results of the group contrast are outlined in Figure 14. No significant differences ($P>0.05$) were observed between the conventionally cured controls and uncured brands containing lactate, and the uncured brands containing lactate versus those uncured brands which did not contain lactate on day 0. Significant differences ($P<0.05$) were observed by day 7 between nitrite added controls and all uncured, no-nitrate-or-nitrite-added brands regardless of whether lactate was included in the ingredient statement or not.

Figure 13. Least squares means of *L. monocytogenes*^a growth differences among uncured, no-nitrate-or-nitrite-added (Brand D-H) and nitrite added (Brand A-C) commercially available deli hams.



^a*L. monocytogenes* growth reported in log CFU/g.

^bBrands: A-C = different brands of commercial nitrite added deli ham product controls and D-H = uncured, no-nitrate-or-nitrite-added commercial deli ham products.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial deli ham products.

^{d-i}Means with different superscripts are different (P<0.05).

Interestingly, no significant differences ($P>0.05$) were observed between the overall growth of uncured brands containing lactate versus those uncured, no-nitrate-or-nitrite-added brands which did contain lactate throughout the challenge study, which further emphasized the variation between the effectiveness of lactate at reduced levels approved for natural and organic products.

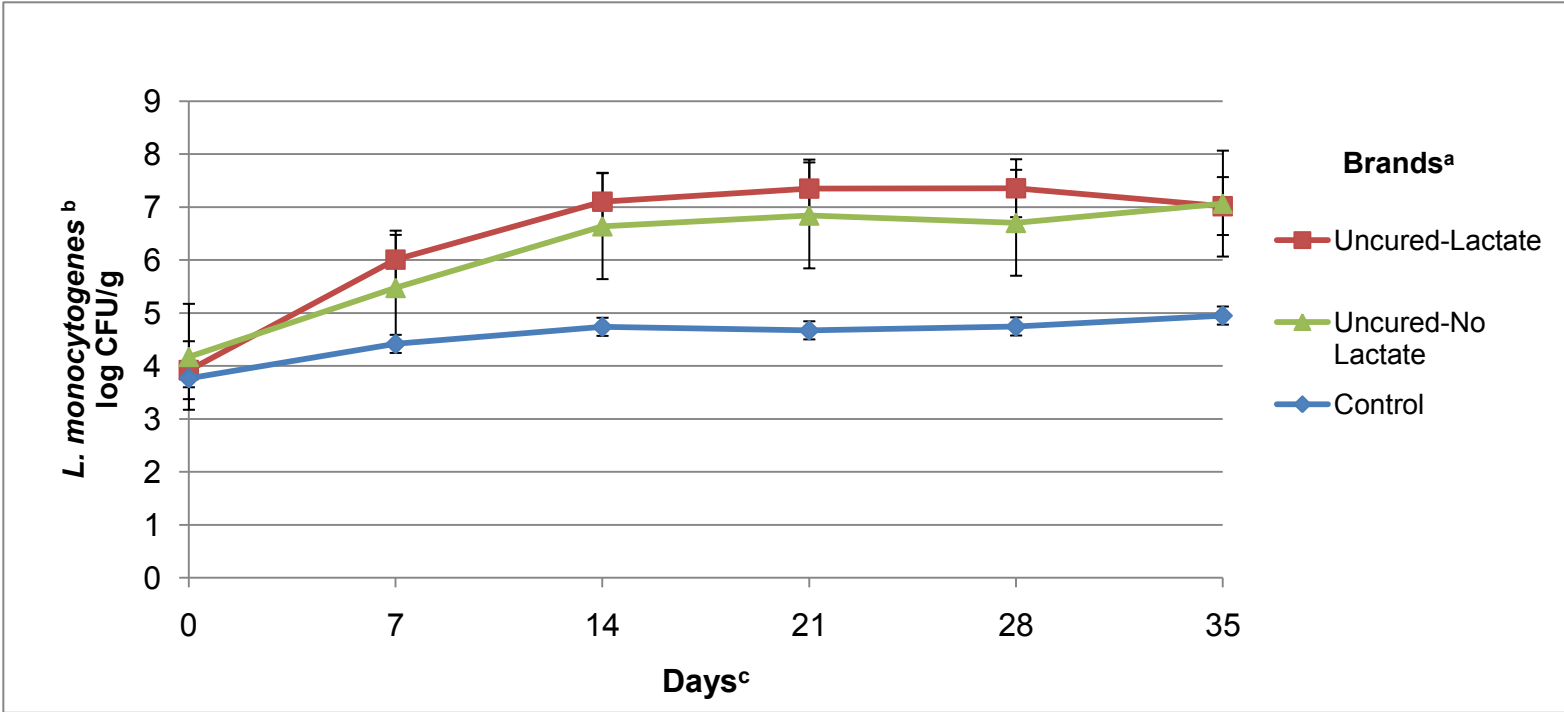
Correlation values between log growth of *L. monocytogenes* and salt, pH, residual nitrate, residual nitrite and water activity (a_w) are reported in Table 6.

Conclusions

Commercially available uncured, no-nitrate-or-nitrite-added frankfurters and deli hams were placed into a challenge study to determine if an increased risk for outgrowth of *Listeria monocytogenes* is associated with these alternatively produced products. Conventionally produced hams and frankfurters were selected for use as comparison to show superior *L. monocytogenes* inhibition through the use of typical industry hurdle technologies including direct nitrite, sodium lactate, and sodium diacetate.

With the exception of Brand I, all uncured, no-nitrate-or-nitrite-added frankfurters were manufactured with the intention to replace nitrite, which resulted in significant concentrations of residual nitrate and nitrite in the meat system. Nevertheless, all uncured, no-nitrate-or-nitrite-added brands were not significantly

Figure 14. Growth of *Listeria monocytogenes* among brands of uncured, no-nitrate-or-nitrite-added (Brand D-H) containing lactate (Brands D and G) or no lactate (Brands E, F and H) and nitrite added (Brand A-C) commercially available deli hams.



^aBrands: D-H = uncured, no-nitrate-or-nitrite-added brands with Brand D and G uncured with lactate and Brands E, F and H uncured no lactate and nitrite added (Brand A-C) commercially available deli ham products.

^bGrowth of *L.monocytogenes* reported in log CFU/g.

^cDays of storage at 10°C over a 35 day study for commercial deli ham products.

Table 6. Pearson's correlation values^a of quality attributes to log growth of *L. monocytogenes* on uncured, no-nitrate-or-nitrite-added (Brand D-H) and nitrite added (Brand A-C) commercially available deli ham products.

Aw	pH	Nitrite	Nitrate	Salt
0.2734**	-0.28487**	-0.17654*	-0.30814**	0.26149

^aR-Values reported

*(P<0.10) trend for correlation to growth of *L. monocytogenes*.

** (P<0.05) significantly correlated to growth of *L. monocytogenes*.

different ($P>0.05$) from at least one of the nitrite added controls with regards to residual nitrite. No correlations were observed for residual nitrate and nitrite levels and their impacts on overall growth of *L. monocytogenes*. Significant differences ($P<0.05$) existed among brands for factors including pH and salt concentration, and while uncured, no-nitrate-or-nitrite-added brands were not different ($P>0.05$) from at least one of the controls, significant correlations ($P>0.05$) were observed for both salt and pH effects on log growth. Increasing sodium chloride concentration resulted in lower ($P<0.05$) levels of *L. monocytogenes* growth while lower pH values resulted in higher growth ($P<0.05$). The negative correlation of pH to log growth was unexpected, and it is suggested that the lack of lactate in the product formulation may have accounted for this correlation. In addition to its abilities to aid in the suppression of pathogens such as *L. monocytogenes*, lactates have the ability to slow the growth of lactic acid producing spoilage bacteria as well. Products which did not contain lactate, reached their maximum population of *L. monocytogenes* by day 14 and at this time competitive inhibition from lactic acid producing spoilage bacteria was ultimately occurring. Uncured, no-nitrate-or-nitrite-added brands which did not contain lactate, had a lower overall pH ($P<0.05$) than those which did.

Factors such as pH, sodium chloride concentration, a_w , residual nitrate, and residual nitrite did not have a definitive influence on the control of *L. monocytogenes* growth. Comparisons among groups of uncured, no-nitrate-or-nitrite-added products revealed that significantly lower ($P<0.05$) levels of overall growth occurred in brands which contained lactate in the formulation. While only reduced levels are permitted

in these products, the lowered levels of lactate added in the meat system allowed for slower growth which was similar to the nitrite added controls, which contained sodium lactate and diacetate. Nevertheless, uncured no-nitrate-or-nitrite-added brands were significantly higher ($P < 0.05$) in growth of *L. monocytogenes* throughout the challenge study.

The influence of sodium lactate addition did not result in significant differences in groups of uncured, no-nitrate-or-nitrite-added deli hams. Nitrite added control brands which contained sodium lactate and sodium diacetate were able to suppress the growth of *L. monocytogenes* and were significantly lower ($P < 0.05$) in overall growth when compared to their no-nitrate-or-nitrite-added counterparts. Reduced levels of lactate addition in uncured, no-nitrate-or-nitrite-added deli hams did not result in significantly lower ($P > 0.05$) levels when compared to uncured products which did not contain lactate.

Variations in a_w , pH, sodium chloride, residual nitrate and residual nitrite were also observed in uncured, no-nitrate-or-nitrite-added deli hams. Negative correlations to growth of *L. monocytogenes* were observed for pH and residual nitrate ($P < 0.05$) indicating that higher levels resulted in lower growth. A_w was positively correlated ($P < 0.05$) to overall log growth of *L. monocytogenes*. Sodium chloride concentrations did not have a significant impact ($P > 0.05$) on growth of *L. monocytogenes*, however there was a trend ($P < 0.10$) for levels of residual nitrite to impact overall growth.

While many of the factors that influence microbial growth are similar to conventionally processed frankfurters and deli hams, uncured, no-nitrate-or-nitrite-added processed meats were unable to suppress the growth of *L. monocytogenes* throughout a 35 day accelerated challenge study. The results of this study indicate that the addition of sodium lactate, even at reduced levels can have an impact on the overall growth of *L. monocytogenes* in uncured, no-nitrate-or-nitrite-added meat products. The impacts of reduced levels of sodium lactate are variable among product categories. Results from the *Lm* challenge study shows that processed meats produced without the direct addition of nitrite and supplemental hurdle technologies (lactate and diacetate) are at an increased risk for *L. monocytogenes* if contamination occurs. The inadequate inhibition of *L. monocytogenes* exhibited by uncured, no-nitrate-or-nitrite-added frankfurters and deli ham in this experiment can be attributed to the modification of non-meat ingredients utilized in the production of these minimally processed meat products. The results of this study indicate that additional antimicrobial measures are needed during the production of natural and organic processed meats in order to provide consumers with the level of safety that is expected of similar conventionally cured meat products.

Acknowledgments

This research was supported through a grant funded by the National Integrated Food Safety Initiative (Grant no. 2006-51110-03609) of the United States

Department of Agriculture Cooperative State Research, Education, and Extension
Service.

References

- [AOAC] Association of Official Analytical Chemists. 1990. Nitrites in cured meat. Official Method 973.31. Official Methods of Analysis. 15th ed. Arlington, Va.; AOAC.
- Ahn DU & Maurer AJ. 1987. Concentration of nitrate and nitrite in raw turkey breast meat and the microbial conversion of added nitrate to nitrite in tumbled turkey breast meat. *Poult Sci* 66:1957-1960.
- Bacus JN. 2006. Natural ingredients for cured and smoked meats. Proceedings of the 59th Annual American Meat Science Association Reciprocal Meat Conference. Champaign-Urbana, Illinois. P 77-78.
- Cassens RG. 1997. Residual nitrite in cured meat. *Food Technol* 51(2):53-55.
- Fox JB, Jr. 1966. The chemistry of meat pigments. *J Agri Food Chem* 14:207-210.
- Hustad GO, Cervený JG, Trenk H, Deibel RH, Kautter DA, Fazio T, Johnston RW & Kolari OE. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in wieners. *Appl Environ Microbiol* 26(1):22-26.
- MacDonald B, Gray JI & Gibbins LN. 1980. Role of nitrite in cured meat flavor: antioxidant role of nitrite. *J Food Sci* 45(4):893-897.
- Radin D, Niebuhr SE & Dickson JS. 2006. Impact of the population of spoilage microflora on the growth of *Listeria monocytogenes* on frankfurters. *J Food Prot* 69:679-681.
- SAS Institute. 2008. SAS/STAT 9.2 user's guide Cary, N.C.: SAS Institute Inc.
- Sebranek JG, Lonergan SM, King-Brink M, Larson E. 2001. Meat science and processing. 3rd ed. Senda, Wis.: Peerage Press. 141p.
- Sebranek JG & Bacus JN. 2007. Cured meat products without direct addition of nitrate or nitrite: what are the issues? *Meat Sci* 77(1):136-147.
- Sindelar JJ. 2006. Investigating uncured no-nitrate-or-nitrite-added processed meat products. Ph.D. Dissertation, Iowa State University. Ames, IA. p 247.
- Sindelar JJ, Cordray JC, Olson DG, Sebranek JG & Love JA. 2007. Investigating quality attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial hams, bacons, and frankfurters. *J Food Sci* 72(8):551-559.

Tompkin RB. 1995. Nitrite. In: Davidson PM, Sofos JN & Branen, AL, editors. Antimicrobials in food. 3rd ed. Boca Raton, FL: CRC Press, Taylor & Francis Group 171-221.

**CHAPTER 4. IMPACT OF NATURAL AND “CLEAN LABEL” ANTIMICROBIALS
ON THE GROWTH OF *LISTERIA MONOCYTOGENES* AND QUALITY
ATTRIBUTES OF UNCURED, NO-NITRATE-OR-NITRITE-ADDED EMULSIFIED
FRANKFURTER STYLE COOKED SAUSAGES**

A paper to be submitted to the Journal of Food Science

Kohl D. Schrader, Joseph C. Cordray, Joseph G. Sebranek, Dennis G. Olson,
Kenneth J. Prusa and Aubrey F. Mendonca

Abstract

With the increased growth in natural and organic processed meats, suppliers have begun to offer “clean label” solutions to improve the safety of minimally processed foods. This study investigated the growth of *Listeria monocytogenes* on uncured, no-nitrate-or-nitrite-added Emulsified Frankfurter Style Cooked Sausages (EFSC) with or without natural or “clean label” antimicrobials, over a 120 d sampling period. Additional quality attributes including residual nitrate, residual nitrite, TBARS analysis and color were also analyzed. No-nitrate-or-nitrite-added treatments with no anti-listerial control measures exhibited a decreased lag time and shorter generation time for initiation of growth, resulting in a greater population of *L. monocytogenes* when compared to the sodium nitrite, sodium lactate/diacetate

controls or alternatively cured products with natural or “clean label” antimicrobials. Curing systems which utilize a natural nitrate source and nitrate reducing starter culture were negatively impacted by the addition of these antimicrobials. Significantly higher levels ($P<0.05$) of residual nitrate and lower levels ($P<0.05$) of residual nitrite were found in treatments which contained natural and “clean label” antimicrobials. Results indicate that natural and “clean label” antimicrobials may serve as an effective intervention strategy for *L. monocytogenes* in the production of uncured, no-nitrate-or-nitrite-added (natural or organic) ready-to-eat (RTE) meat products, but may have negative impacts on the generation of nitrite for curing reactions and other quality attributes.

Introduction

Listeria monocytogenes, the pathogen responsible for listeriosis, has emerged as a serious problem in ready-to-eat (RTE) processed meat products. While the occurrence of listeriosis is rare, the illness carries one of the highest mortality rates (~30%) of any foodborne pathogen (Jay and others 2005). In response to outbreaks of foodborne listeriosis, as well as recalls of meat and poultry products adulterated by *L. monocytogenes*, the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) established a strict “zero tolerance” policy for the presence of *L. monocytogenes* in RTE meat and poultry products. As a result, processors must employ one of three alternatives to control *L. monocytogenes* in RTE meat products. (Code of Federal Regulations

2003). Of these alternatives, organic acids including lactates and diacetates are most commonly utilized as an antimicrobial ingredient in the production of cured meat products, and have been found to provide significant protection against foodborne pathogens including *L. monocytogenes* (Maas and others 1989; Glass and Doyle 1989b; Lianou and others 2007). Despite the well established impacts on food safety, the use of preservatives, including antimicrobials such as lactate and diacetate, are not permitted in uncured, no-nitrate-or-nitrite-added, natural or organic, meat products. These products make use of naturally occurring nitrates and nitrites found in vegetables, to produce products that have typical cured meat characteristics including color and flavor (Sindelar and others 2007a). Even so, the cured meat properties achieved in these products are highly variable and have raised concerns for the overall safety of natural and organic RTE processed meats (Sebranek and Bacus 2007). Previous work indicates that the modifications in ingredients, including lower levels of nitrite and lack of antilisterial controls, puts uncured, no-nitrate-or-nitrite-added RTE meats at an increased risk for *Listeria monocytogenes* and alternative control measures are warranted.

The objective of this study was to determine if natural and “clean label” antimicrobials can serve as a possible hurdle technology for meat processors producing uncured, no-nitrate-or-nitrite-added processed meats, and what quality attributes may be effected by the addition of these ingredients into alternative curing systems.

Materials and Methods

Experimental Design and Statistical Analysis

Statistical analysis was performed for all measurements using the Statistical Analysis System (SAS Institute 2008). The main plot consisted of 3 blocks (replication) and 8 EFSC sausage treatments resulting in 24 observations for microbial analysis and proximate composition. The model included the fixed main effects of treatment, replication, day, and the interaction of treatment x day.

Within the main factorial design was a split plot for measurements over time. The split plot contained 5 sampling periods (day 0, 14, 28, 56, 90) and combined with the main plot resulted in a total of 120 observations for color, nitrate, nitrite, pH, lipid oxidation, water activity and microbial analysis. The random effect was the interaction of treatment x replication. Contrast comparisons and correlations were also performed to determine factors that were contributing to microbial growth.

The significant main effect means for all experiments were separated and least significant differences were found using Tukey-Kramer multiple pairwise comparison method. Significance was determined at $P < 0.05$.

Product Procurement and Manufacture

Ready-to-Eat EFSC sausages were formulated using methods described by Sindelar (2006a). 80% lean beef trimmings were obtained from the Iowa State University Meat Laboratory (Ames, Iowa, U.S.A.) and 60% lean pork trimmings were

obtained from Potthoff Foods Inc. (Des Moines, Iowa, U.S.A.). The 80% lean beef trimmings and 60% lean pork trimmings were ground (Biro MFG Co., Marblehead, Ohio, U.S.A.) using a 9.5mm and 1.27mm plate respectively. Fat content was determined by taking 5.9kg samples and performing analysis using a Anyl-Ray Fat Analyzer (Kartrig Pak, Model 316-48, Davenport, Iowa, U.S.A.). All treatments were formulated to obtain a finished lean content of 30%. Trimmings were separated into eight batches (13.61 kg each) and treatments (TRT 1-6) and controls (C+ and C-) were randomly assigned to the batches. All treatments (TRT 1-6) and controls (C+ and C-) were manufactured using the same base formulation that included the following ingredients: 39.67% beef trimmings, 39.67% pork trimmings, 15.87% ice/water, 1.79% salt, 1.59% dextrose, 1.42% spices (Blend TG-05-405-000 (mustard, spices, garlic powder) A.C. Legg Packing Co., Calera, Ala., U.S.A.). TRT 1, TRT 2, and TRT 3 contained 0.20% vegetable juice powder (VegStable 502, Florida Food Products, Inc., Eustis, Fla., U.S.A.), and 0.256% starter culture containing *Staphylococcus carnosus* (CS 299 Bactoferm™, Chr. Hansen Inc., Gainesville, Fla., U.S.A.). TRT 4, TRT 5, and TRT 6 contained 0.45% vegetable juice powder (VegStable 504, Florida Food Products, Inc., Eustis, Fla., U.S.A.). Furthermore, TRT 2 and TRT 5 also contained a natural antimicrobial blend of cherry powder, lemon powder and vinegar at 1.4% (VegStable 507C+, Florida Food Products, Inc., Eustis, Fla., U.S.A.), and TRT 3 and TRT 6 also contained a clean label antimicrobial blend of cultured corn sugar and vinegar at 3% (VerdadNV55, Purac America, Lincolnshire, Ill., U.S.A.). The positive control (C+) contained

0.0436% sodium erythorbate and 0.0124% sodium nitrite and 2.5% potassium lactate/sodium diacetate (OptiformPD.4, Purac America, Lincolnshire, Ill., U.S.A.). The negative control (C-) was the base formulation with no additional ingredients added. No phosphates were included in any of the formulations, as phosphates are not permitted in natural and organic meat products and these products were intended to be similar to that category of products.

Emulsions were produced using methods as outlined by Rust (1987). EFSC sausages were manufactured using a vacuum bowl cutter (Krämer & Grebe Model VSM65, Krämer & Grebe GmbH & Co. KG., Biendenkopf-Wallau, Germany). The beef trim was chopped with salt vegetable juice powder (or nitrite depending upon treatment) and half of ice/water under vacuum until 3°C was achieved. The bowl cutter was scraped and the pork, dextrose, spices, starter culture or sodium erythorbate (depending upon treatment), appropriate antimicrobial (depending on treatment) and remaining water and ice were added. The product was chopped under vacuum until 14°C was reached. After chopping the batter was transferred to a rotary vane vacuum-filling machine with linking attachment (Risco vacuum stuffer, Model RS 4003-165, Stoughton, Mass., U.S.A.) and stuffed into 33mm impermeable plastic casings (WP-E Clear 35 Micron, WorldPac USA International, Sturtevant, Wis., U.S.A.). The casings had an O₂ permeability rate of 67 cm³/m² per 24 h at 1 atm and a water vapor permeability of 130 g/m² per 24 h. Impermeable casings were used to control cross-contamination effects that any environmentally released nitric oxide gas could have on the treatments during thermal processing.

TRTs were placed on separate smokehouse trucks to allow for application of appropriate thermal processing cycles. The EFSC sausages were transferred to two single truck thermal processing ovens (Maurer, AG, Reichenau, Germany; Alkar, Model MT EVD RSE 4, Alkar Engineering Corp., Lodi, Wis., U.S.A.). TRT 1, TRT 2, and TRT 3 began an incubation period when the internal temperature of the EFSC sausages reached 37.8°C. Incubation was conducted at 40.6°C dry bulb and 39.4°C wet bulb temperatures. Cooking was performed using a common frankfurter smokehouse schedule reaching an internal temperature of 71.1°C. TRT 4, TRT 5, TRT 6, C+ and C- did not undergo an incubation period, only the frankfurter schedule the aforementioned treatments received. After thermal processing the EFSC sausages were chilled for 12 h at 0-2°C. The EFSCS were peeled and placed in barrier bags (Cryovac B540, Cryovac Sealed Air Cor., Duncan, S.C., U.S.A.) and vacuum packaged. The packaging film had an O₂ transmission rate of 3-6 cc/m² per 24 h at 1 atm, 4.4°C and 0% RH and a water vapor permeability of 0.5-0.6 g/645cm² per 24 h and 100% RH.

Proximate Analysis

Proximate composition was determined on day 0 including crude fat (ether extract method, AOAC 1990a), moisture (air oven drying method, AOAC 1990b) and crude protein (combustion method, AOAC 1993).

Water Activity

Available moisture was determined using a water activity meter (AquaLab 4teDecagon Devices Inc., Pullman, Wash., U.S.A.). Measurements were obtained on day 0, 14, 28, 56, and 90 and were performed in duplicate.

Sodium Chloride Analysis

Sodium Chloride content was determined using the Quantab Method as described by Sebranek and others (2001). Ground samples were analyzed in duplicate on day 0 using titrating strips (Quantab Chloride Tirator, Environmental Test Systems, Inc., Elkhart, Ind., U.S.A.).

Color Measurements

Color measurements were performed using a Hunterlab Labscan spectrophotometer (Hunter Associated Laboratories Inc., Reston, Va., U.S.A.). The colorimeter was calibrated using the same packaging material as used on the samples and placed over the white standard tile. Values for the white standard tile were $X=81.72$, $Y=86.80$ and $Z=91.46$. Internal color of the frankfurters was measured after slicing the frankfurters lengthwise and immediately measuring the internal color. Illuminant A, 10° standard observer with a 1.27 cm viewing area and a 1.78 cm port size was used to evaluate frankfurter samples. Commission International d'Eclairage (CIE) L^* (lightness), a^* (redness), and b^* (yellowness) was determined by reflectance ratio of wavelengths 650/670 nm (Hunt and others 1991).

Measurements were taken at 3 randomly selected areas on the samples (3 for internal, 3 for external) in duplicate, and the resulting average was used in data analysis.

pH Determination

The pH was measured throughout the production period with a pH meter (HI 99161, Hanna Instruments, Woonsocket, R.I., U.S.A.) equipped with a pH probe (FC202D, Hanna Instruments, Woonsocket, R.I., U.S.A.) calibrated with standard buffers at pH 4.0 and pH 7.0. Measurements of pH were performed for each treatment on the raw meat blocks, at stuffing and on the frankfurters after the incubation period. The pH was determined by inserting the probe directly into the meat itself.

The pH of the frankfurters was measured on the finished product at day 0, 14, 28, 56, and 90 and samples were prepped by first blending the ground EFSC sausage samples with distilled, de-ionized water in a 1:9 ratio, then measuring the pH with a pH/ion meter (Accumet 925: Fisher Scientific, Fair Lawn, N.J., U.S.A) equipped with an attached electrode (Accumet Flat Surface Epoxy Body Ag/AgCl combination Electrode Model 13-620-289, Fisher Scientific, Fair Lawn, N.J., U.S.A) calibrated with phosphate buffers 4.0 and 7.0, according to the method of Sebranek and others (2001). For each treatment measurements were performed in duplicate.

TBARS Analysis

Lipid oxidation was measured by the modified 2-thiobarbituric acid reactive substance (TBARS) test as described for cured meats (Zisler and Watts 1962). TBARS values were reported as mg of malonaldehyde equivalents/kg of meat sample. Treatments were measured at day 0 (after packaging), 14, 28, 56, 90 and each treatment was measured in duplicate.

Residual Nitrite Analysis

Residual nitrite was determined by utilizing the AOAC method (AOAC 1990). The same finely chopped/ground samples that were used for water activity, TBARS and pH determination were used for the residual nitrite measurement. All assays were executed in duplicate.

Residual Nitrate Analysis

Samples were collected during the manufacture of EFSC sausages throughout processing and on days 0, 14, 28, 56, and 90. Each sample was frozen and stored at -5°C until further evaluation could be performed. Methods for residual nitrate analysis were modifications of Ahn and Maurer (1987). Five g of meat product samples were weighted in a 50 ml test tube and homogenized with 20 ml of distilled, de-ionized water using a Polytron homogenizer (Type PT 10/35, Brinkman Instruments Inc., Westbury, N.Y., U.S.A.) at high speed for 10 s. The homogenate was then heated for 1 h in an 80°C water bath. Following cooling in cold water for

10 min, 2.5 ml of the homogenate was transferred to a disposable test tube (16 x 100 mm). Carrez II (dissolve 10.6 g potassium ferrocyanide in 100 ml of distilled, de-ionized water) and Carrez I (dissolve 23.8 g of zinc acetate in 50 ml of distilled, de-ionized water, then add 3 ml of glacial acetic acid and dilute with distilled, de-ionized water to 100 ml) reagents were added (0.1 ml each) to precipitate proteins. The solution was diluted with 2.3 ml of distilled, de-ionized water and mixed thoroughly. Following precipitation, the supernatant was centrifuged at 10,000 x g for 20 min and the clear supernatant was used for nitrate analysis. Analysis was performed using high performance liquid chromatography (Agilent 1100 Series HPLC System, Agilent Technologies, Wilmington, Del., U.S.A.) with an Agilent Zorbax SAX column (analytical 4.6 x 150 mm, 5-micron) (Agilent, Wilmington, Del., U.S.A.). The elution buffer was 15mM phosphate buffer, pH of 2.35, with isocratic elution. The flow rate was 1.0 ml/min and the sample volume was 25 µL. A 210 nm wavelength was utilized and the area of the nitrate peak was used to calculate nitrate concentration using a nitrate standard curve. Results were reported in parts per million (ppm).

Microbial Challenge Study

A five-strain cocktail mixture of *Listeria monocytogenes* cultures was used in the study and contained: *L. monocytogenes* Scott A, H7764 1/2a, H7969 4b, H7962 4b, and H7762 4b. Cultures were individually grown in trypticase soy broth containing 0.6% yeast extract (TSB-YE broth) (Difco, Becton, Dickson and

Company, Sparks, Md., U.S.A.) for 24 h at 35°C. Following incubation, 1 ml of each culture was added to 500 ml of TSB-YE broth and incubated for an additional 24 h at 35°C. The original cocktail was diluted using sterile 0.1% peptone water (Difco, Becton Dickson and Company, Sparks, Md., U.S.A.) to target for an inoculum level of 3-log CFU/g on the EFSCS.

Whole EFSC sausages were aseptically removed from the package and surface inoculated with 1 ml of a 5-strain cocktail mixture of *L. monocytogenes*. Samples were hand massaged for 10-15 seconds to distribute microorganisms, vacuum sealed and stored at 4°C for 120 days. Evaluations were performed weekly for the first six weeks and bi-weekly for the remainder of the study. Samples were prepared in duplicate by first blending whole frankfurters with sufficient 0.1% peptone water to achieve a 1:5 dilution of each sample. Appropriate dilutions were then plated on modified oxford media (MOX) and incubated at 35°C for 48 hours to allow for enumeration of *L. monocytogenes*.

Results and Discussion

Product Processing Attributes

A variety of product and processing parameters were recorded throughout the manufacture of the uncured, no-nitrate-or-nitrite-added EFSC sausages. Means for beef trim were as follows: 12.37% fat, pH of 5.57 and a temperature of -0.44°C. The means for pork trim attributes were as follows: 58.93% fat, pH of 6.00 and a temperature of -0.761°C. pH of all TRTs including the controls (C- and C+) were

measured at stuffing and significant differences ($P < 0.05$) were found between TRTs and are outlined in Figure 1. The pH was also measured after the incubation step for TRT 1, TRT 2, and TRT 3. pH measurements were 5.58, 5.81, and 5.57 respectively and no significant differences ($P > 0.05$) were observed.

Sodium Chloride

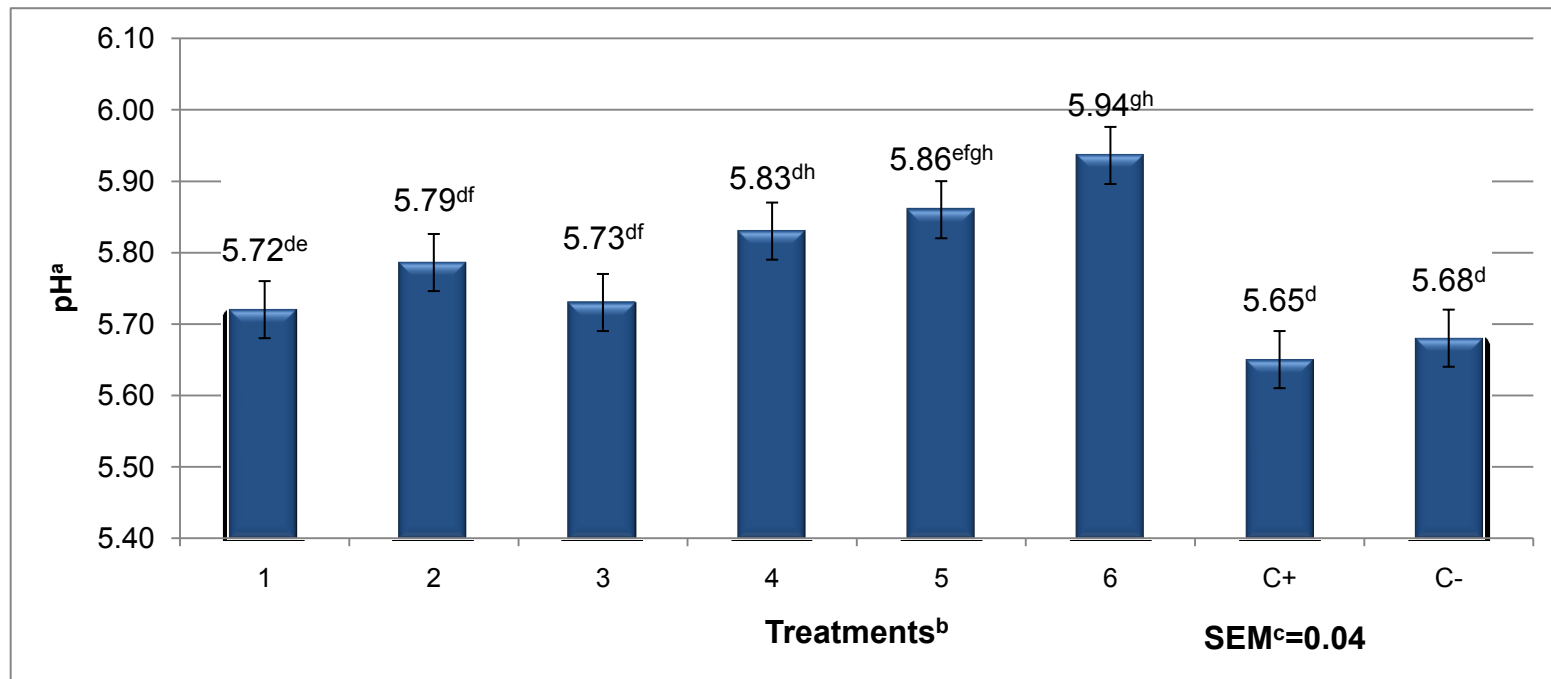
No significant differences ($P > 0.05$) in sodium chloride content were found among treatments and values ranged from 2.21 to 2.41 with a standard error of 0.01 indicating that all treatments were consistent in salt concentration. No correlation ($P > 0.05$) was observed for salt concentration to log growth of *L. monocytogenes*.

Color Measurements

Internal color measurements were measured for EFSC sausages using the Commission International d'Eclairage (CIE) L^* (lightness), a^* (redness), and b^* (yellowness) and was determined by reflectance ratio of wavelengths 650/670 nm (Hunt and others 1991). Neither a significant treatment, day, nor significant effect of the interaction between the two ($P > 0.05$) was observed for internal CIE L^* values.

No interaction was present for treatment*day for internal CIE b^* values but the main effect of treatment was significant and the corresponding least squares means for TRTS (1-6) and Controls (C+, C-) are reported in Figure 2. TRT 2 and 5 had significantly higher b^* values than all other treatments including the nitrite added

Figure 1. Least squares means of pH^a differences among uncured, no-nitrate-or-nitrite-added (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.



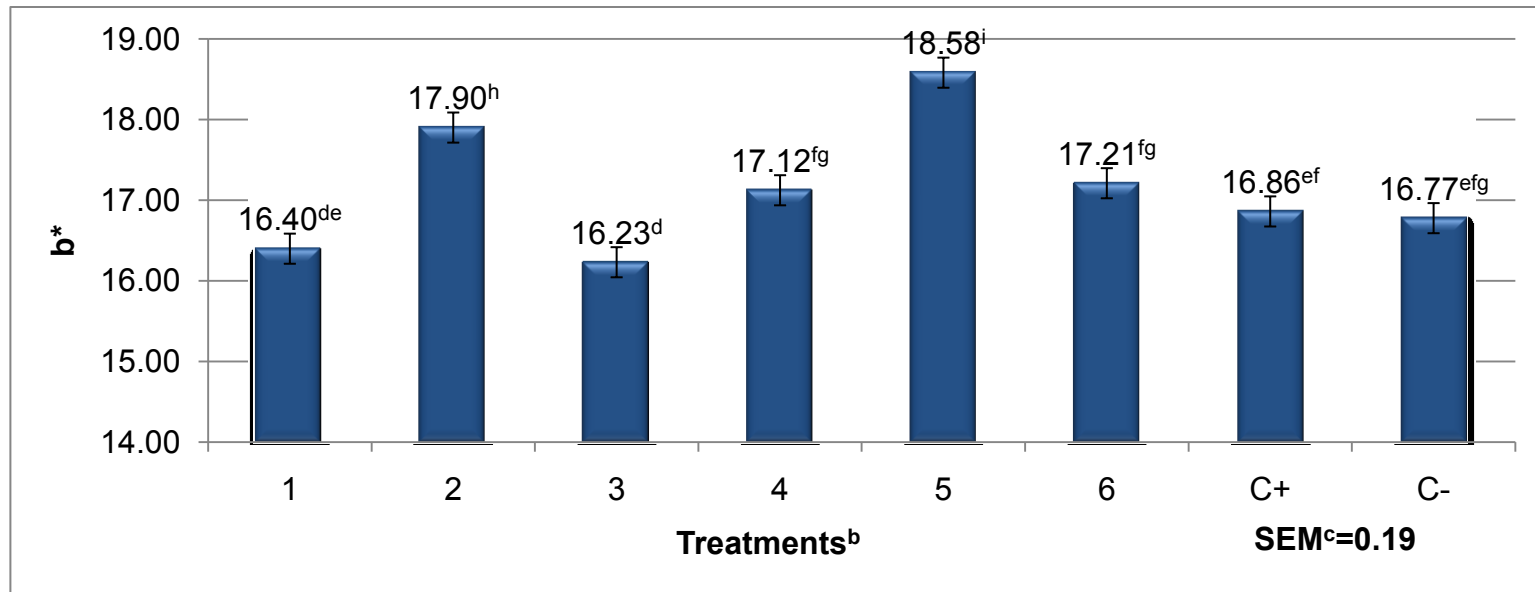
^apH of EFSC sausage samples.

^bTreatments (TRT): 1-4 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages and C+ = nitrite added control and C- = uncured control.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and uncured and nitrite added controls.

^{d-h}Means with different superscripts are different (P<0.05).

Figure 2. Least squares means for the main effect of treatment for objective color (b^*)^a differences among uncured, no-nitrate-or-nitrite-added (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.



^aCommission International D'Eclairage (CIE) L* a* b* where L*= lightness, a* = redness, and b* = yellowness on a 0-100 white scale. E

^bTreatments (TRT): 1-4 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages and C+ = nitrite added control and C- = uncured control.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added (TRTs 1-6) and uncured (C-) and nitrite added (C+) controls.

^{d-i}Means with different superscripts are different (P<0.05).

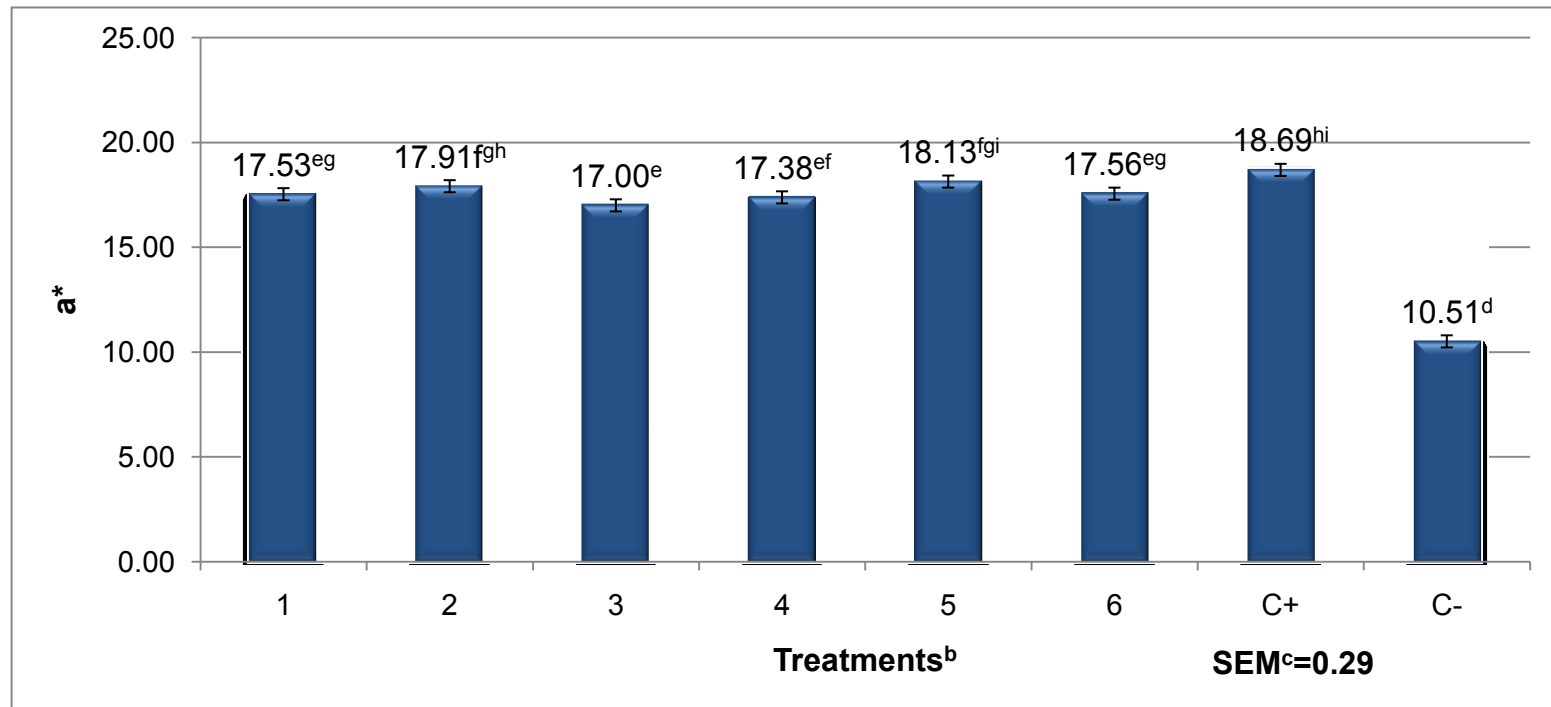
control (C+) indicating a greater yellowness of the product, which could be an effect of the added antimicrobial on these treatments.

A significant ($P<0.05$) effect of TRT as well as day was observed for CIE a^* redness values. TRTs 2 and 5 were not significantly different from the nitrite added control (C+) indicating a comparable amount of cured meat color was present. CIE a^* values generally decreased overtime with the largest significant decreased ($P<0.001$) between day 0 and day 90. Least squares means for the effects of TRT can be observed in Figure 3. A significant positive correlation ($P<0.05$) was observed for nitrite as well as nitrate for a^* values, which would be expected as a higher level of residual nitrate and nitrite would produced more cured color and in turn higher a^* values.

Proximate Composition

Proximate Composition for EFSC sausages for moisture ranged from 61.32% to 61.92% while fat values ranged from 20.44% to 21.46% and there were no significant differences observed for moisture or fat ($P>0.05$). Significant differences were observed for protein content, and TRTs 3 and 6 tended to have lower protein values when compared to all other TRTs including the nitrite added control, while TRTs 1, 2, 4, and 5 were not significantly different from the nitrite added control (C+). This could be a result of the selected antimicrobial as it was the same in both TRTs. Least squared means values are reported for moisture, fat and protein

Figure 3. Least squares means for the main effect of treatment for objective color (a^*)^a differences among uncured, no-nitrate-or-nitrite-added (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.



^aCommision International D'Edairerage (CIE) L* a* b* where L*= lightness, a* = redness, and b* = yellowness on a 0-100 white scale.

^bTreatments (TRT): 1-4 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages and C+ = nitrite added control and C- = uncured control.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added (TRTs 1-6) and uncured (C-) and nitrite added (C+) controls.

^{d-i}Means with different superscripts are different (P<0.05).

Table 1. Least squares means for proximate composition of uncured, no-nitrate-or-nitrite-added (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.

TRT ^a	Moisture%	Fat%	Protein%
1	61.12 ^c	21.31 ^c	12.99 ^{de}
2	60.44 ^c	21.19 ^c	12.89 ^{ce}
3	61.38 ^c	20.44 ^c	12.49 ^c
4	60.77 ^c	21.38 ^c	13.05 ^{de}
5	61.02 ^c	20.54 ^c	13.02 ^{de}
6	60.32 ^c	21.46 ^c	12.61 ^{cd}
C+	60.97 ^c	21.07 ^c	12.98 ^{de}
C-	61.92 ^c	20.61 ^c	13.23 ^e
SEM^b	0.64	0.90	0.03

^aTreatments (TRT): 1-4 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages and C+ = nitrite added control and C- = uncured control.

^bSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added (TRTs 1-6) and uncured (C-) and nitrite added (C+) controls.

^{c-e}Means within column with different superscripts are different (P<0.05).

analysis in Table 1. Despite the slight differences in protein values, these results show that all treatments were uniform in composition.

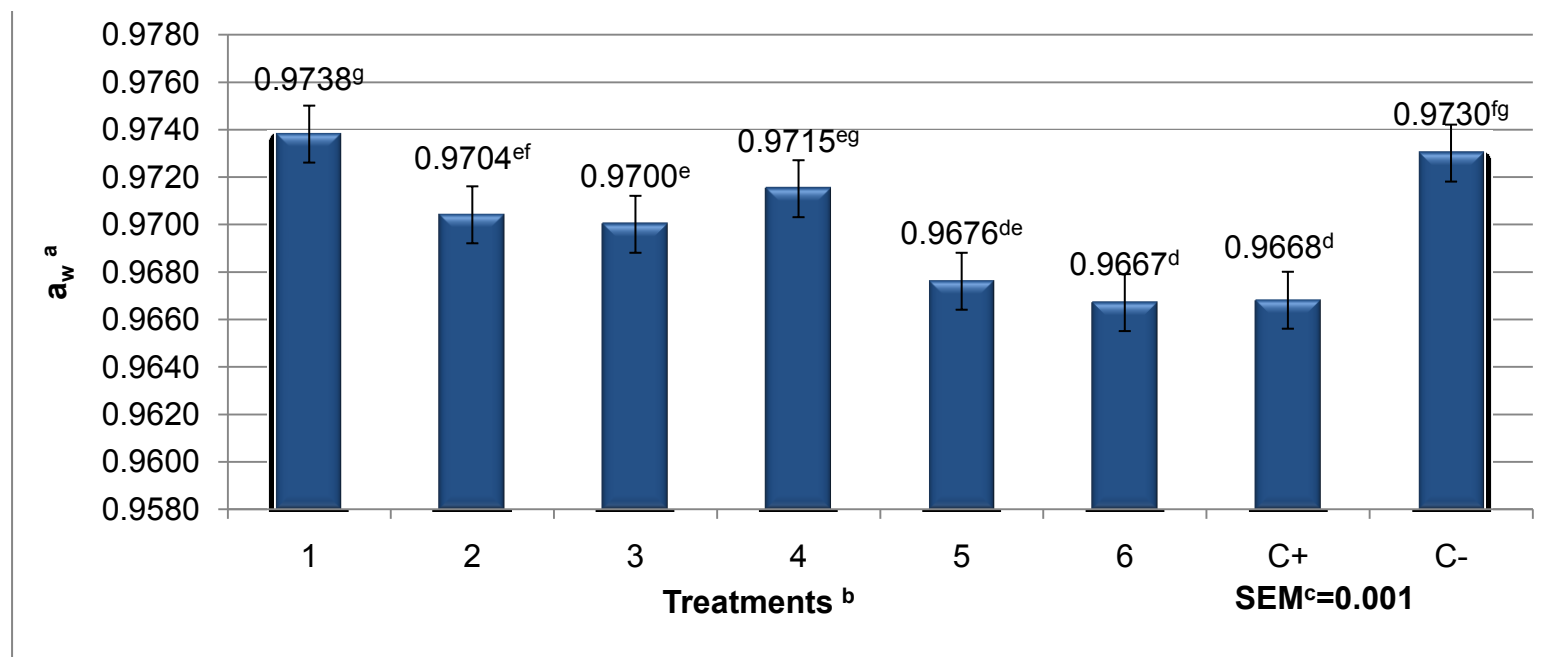
Water Activity (a_w)

No significant differences ($P>0.05$) were detected for the main effects of day or treatment*day interaction. Significant differences ($P<0.05$) did exist for the main effects of treatment and the least square means are displayed in Figure 4. TRTs 5 and 6 were not significantly different from the nitrite added control, while all other treatments were significantly higher in a_w readings ($P<0.05$). There was a positive correlation ($P<0.05$) observed for water activity to log growth of *L. monocytogenes*, indicating a lower a_w would result in lower overall growth, which was the case in these treatments.

pH Determination and TBARS Analysis

No significant differences were observed for the treatment*day interaction for pH, however, the main effect of treatment was significant. Least squares means of pH for treatments are presented in Figure 5. TRTs 4, 5 and 6 were significantly higher in pH ($P<0.05$) than all other treatments except TRT 2. The 0.02-0.03 pH unit difference of TRTs 4, 5 and 6 may be a result of the preconverted powder that was utilized in the production of these uncured, no-nitrate-or-nitrite-added meat products. The pH of the preconverted, nitrite containing vegetable powder ranges from 7.5 to 9.5 which is 1-2 pH units higher than the nitrate containing vegetable powder. The pH effect in the finished product mirrors the pH that was observed at stuffing. There

Figure 4. Least squares means for the main effect of treatment for a_w ^a differences among uncured, no-nitrate-or-nitrite-added (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.



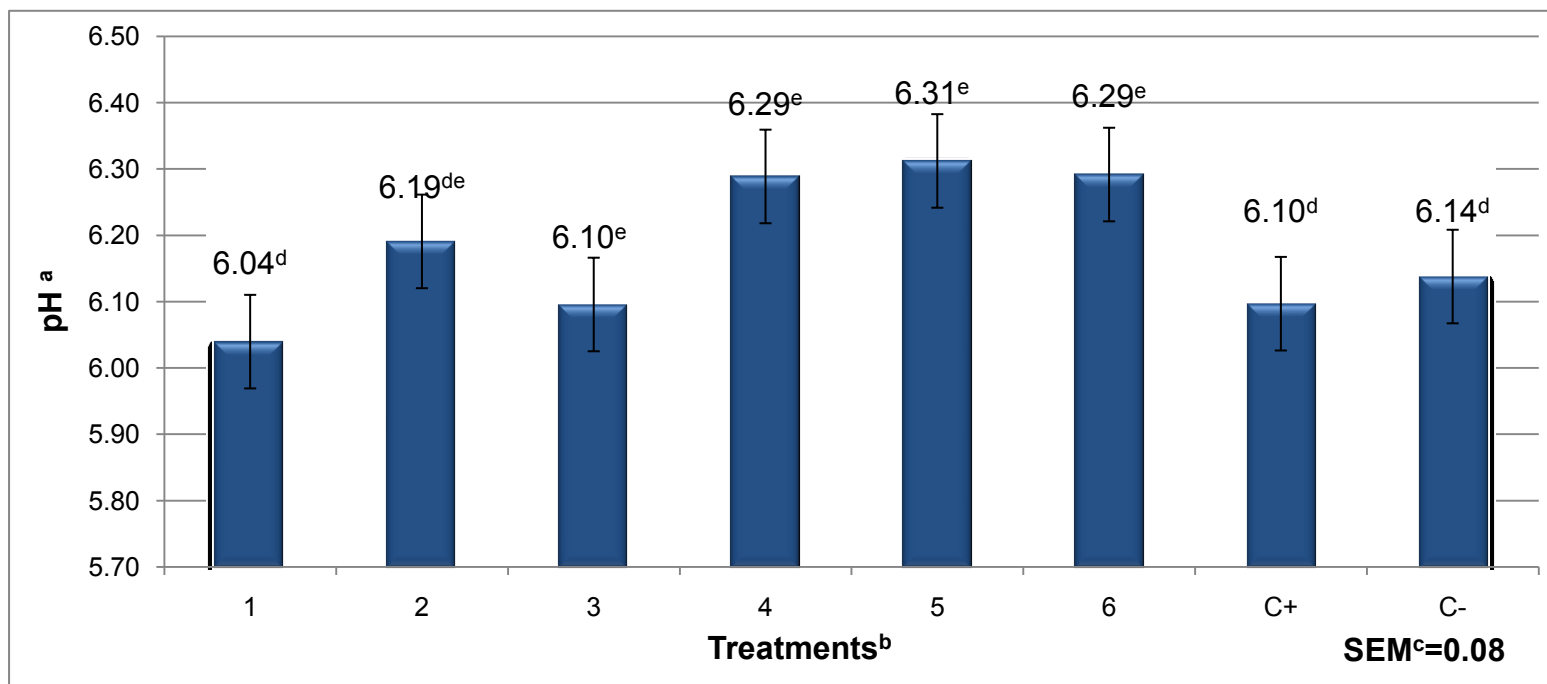
^aWater activity determination reported as ratio of the vapor pressure of water in a material to the vapor pressure of pure water.

^bTreatments (TRT): 1-4 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages and C+ = nitrite added control and C- = uncured control.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added commercial frankfurter products.

^{d-g} Means with different superscripts are different (P<0.05).

Figure 5. Least squares means for the main effect of treatment for pH^a differences among uncured, no-nitrate-or-nitrite-added (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.



^apH of EFSC sausage samples.

^bTreatments (TRT): 1-4 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages and C+ = nitrite added control and C- = uncured control.

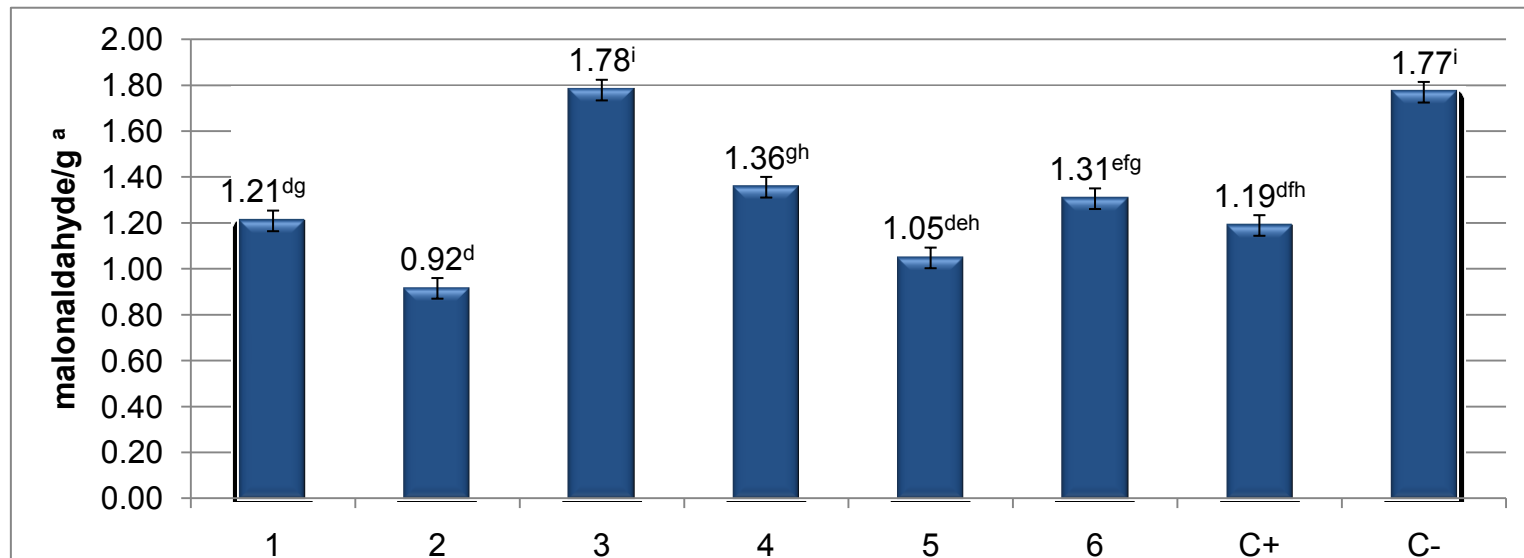
^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added EFSC sausages.

^{d-e} Means with different superscripts are different (P<0.05).

was no significant correlation ($P>0.05$) of pH to log growth of *L. monocytogenes*, however there was a trend ($P<0.10$) for a negative relationship between pH and water activity, indicating that a higher pH would result in a lower water activity. As previously described a lower water activity would result in a lower growth of *L. monocytogenes*, indicating that a higher pH may in fact be more desirable for safety purposes.

Significant differences ($P<0.05$) were observed for the main effect of treatment for lipid oxidation measured by TBARS while no treatment*day interaction occurred. TBARS values ranged between 0.92 and 1.78 which are reaching the levels for detectable lipid oxidation. A TBARS value of 0.05 to 1.0 is considered to be the threshold for oxidized odor and 1.0 to 2.0 for oxidized flavor (Tarladgis 1962). TBARS analysis revealed that the pork and beef trimmings utilized for the production of the EFSC sausages were already experiencing high levels of oxidation occurring with TBARS values of 1.77 and 2.90 for beef and pork trimmings respectfully. Despite the high TBARS values that occurred at Day 0 and throughout the 90 day storage period, significant differences in treatments revealed a negative correlation ($P<0.05$) of TBARS values to residual nitrite, indicating that a higher level of residual nitrite would result in lower corresponding TBARS values. This would be expected as nitrite is known to be a strong antioxidant and effective in controlling oxidative rancidity. Based on the results of the least squared means shown in Figure 6, Treatment 3 was significantly higher in TBARS value across all other treatments

Figure 6. Least squares means for the main effect of treatment for lipid oxidation (TBARS)^a of uncured, no-nitrate-or-nitrite-added (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.



^aTBARS values of EFSC sausage samples reported in malonaldehyde/g.

^bTreatments (TRT): 1-4 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages and C+ = nitrite added control and C- = uncured control.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added EFSC sausages.

^{d-e} Means with different superscripts are different (P<0.05).

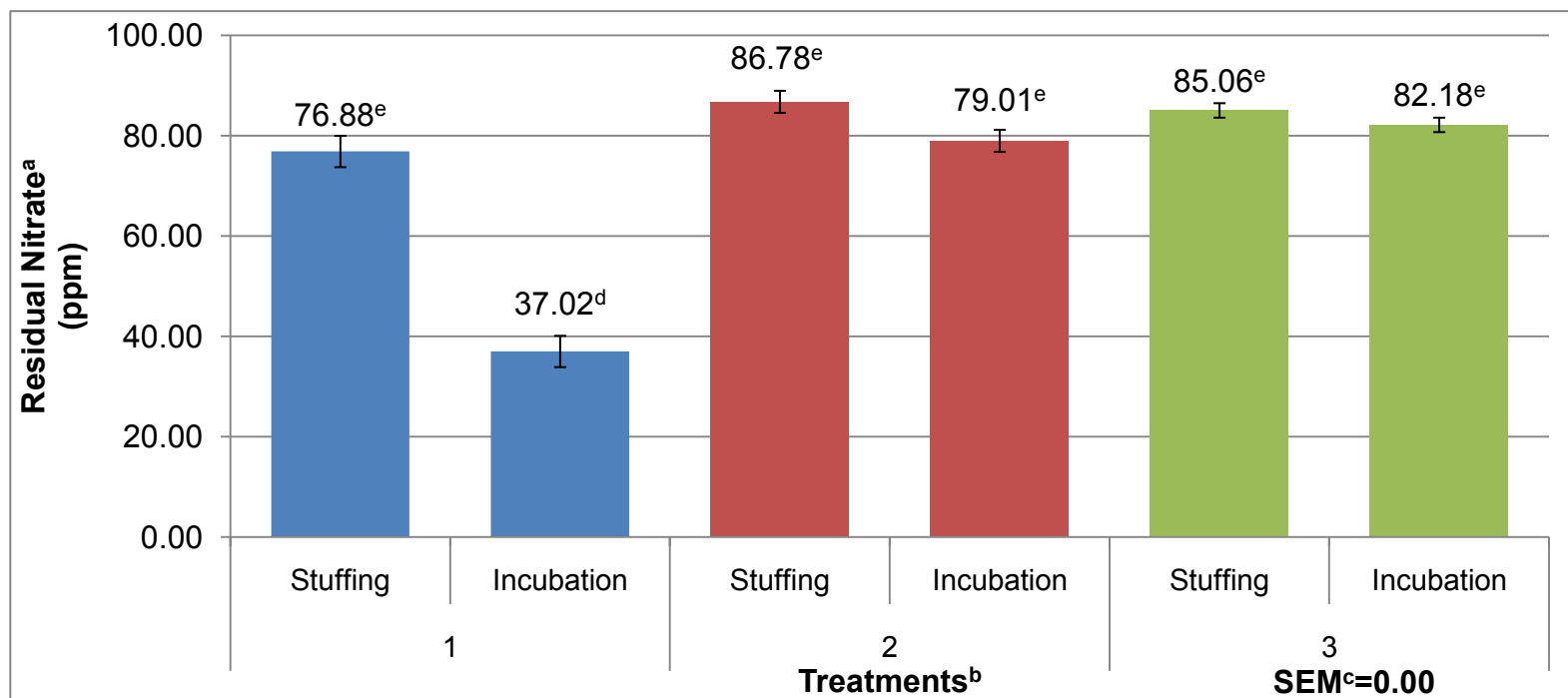
except for the uncured, negative control (C-). Interestingly, TRT 3 had the lowest level of ingoing nitrite among all other treatments except for the uncured control.

Residual Nitrate Analysis

Residual nitrate analysis was determined at stuffing, post-incubation (depending upon treatment) and throughout a 90-day storage period. TRTs 1, 2, and 3 were formulated with a nitrate containing vegetable powder, and the pre-incubate levels would be expected to be remarkably higher than Treatments 4, 5, and 6 which were formulated with a nitrite containing vegetable powder. All treatments which contained the nitrate containing powder were significantly higher in residual nitrate than all other treatments ($P < 0.05$) and residual nitrate was not different in treatments 4, 5, and 6 when compared to the nitrite added control (C+). The negative control (C-) was significantly lower than all treatments.

Post incubation residual nitrate was significantly lower ($P < 0.05$) in Treatment 1 when compared to Treatments 2 and 3. There was no significant difference ($P > 0.05$) between the residual nitrate levels of Treatments 2 and 3 after the incubation period. The high levels of residual nitrate post incubation in Treatments 2 and 3 which contained added antimicrobials, indicate that an insufficient amount of nitrate was converted in the incubation period. The comparison of residual nitrate at stuffing and after the incubation period (Figure 7) demonstrates that there was little

Figure 7. Least squares means differences in residual nitrate^a post incubation of uncured, no-nitrate-or-nitrite-added (TRT 1-3) EFSC sausages.



^aResidual nitrate reported in ppm.

^bTreatments (TRT): 1-3 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages.

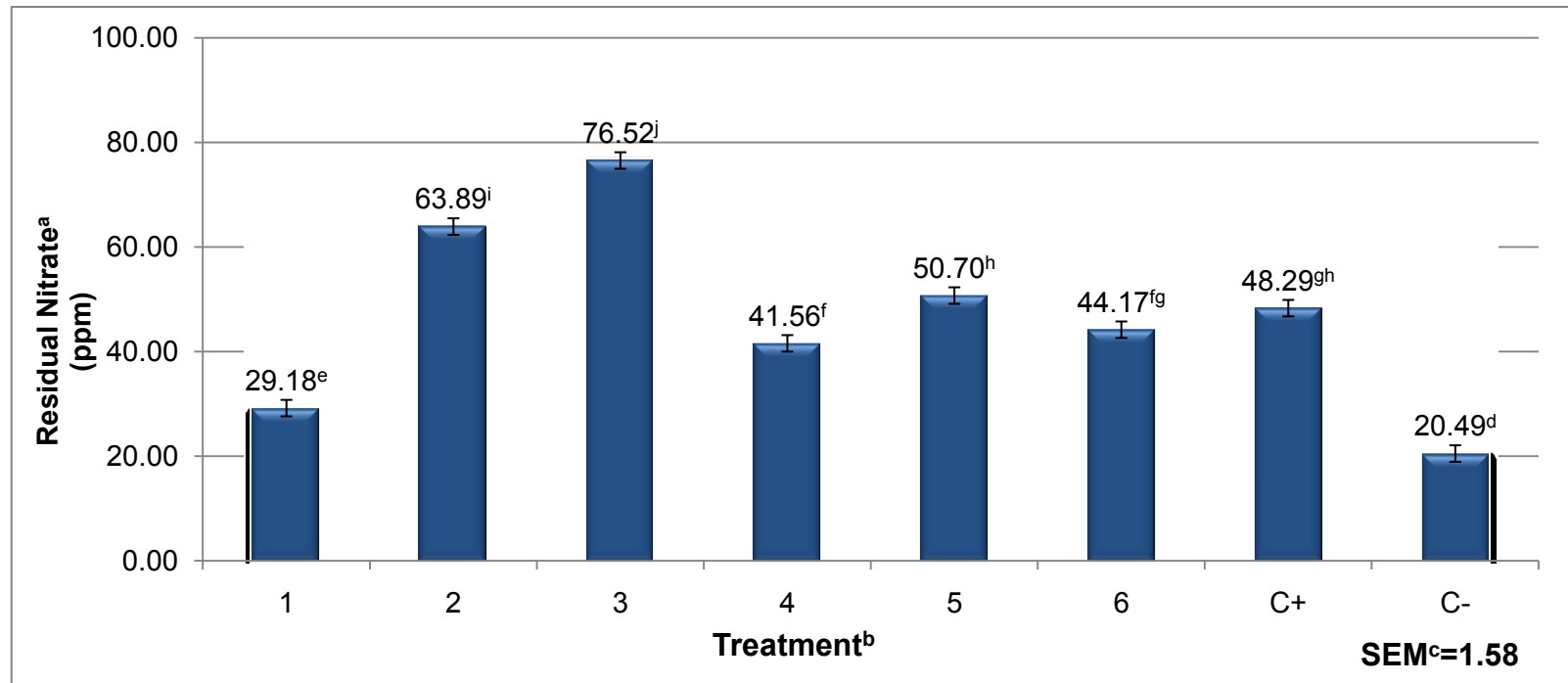
^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added EFSC sausages.

^{d-e} Means with different superscripts within TRT are different (P<0.05).

to no conversion, and thus no significant difference in residual nitrate levels after the incubation period in TRTs 2 and 3, leading an indication that the added antimicrobials may have interfered with the nitrate reductase activity of the starter culture. Sindelar and others (2007b) emphasized the importance of an incubation period in EFSC sausage to allow for conversion of nitrate to nitrite to take place by the added starter culture. The addition of natural and clean label antimicrobials appears to have a negative impact on starter culture activity and may require additional time in the incubation period to allow the starter culture to reach its stationary phase and begin reducing nitrate to nitrite.

No significant ($P>0.05$) difference for the interaction of treatment*day was observed for residual nitrate, however, as expected significant differences were observed for the main effects of treatments and the least squares means for treatment are displayed in Figure 8. Treatments 2 and 3 exhibited the highest level of residual nitrate when compared to all other treatments and where significantly different from each other as well ($P<0.05$). Antimicrobial added to Treatment 2 may have been cause for this significant difference as it contained cherry powder, which is a natural form of ascorbate. These low levels of a naturally occurring curing accelerator may have allowed for greater conversion of nitrate to nitrite and subsequently nitric oxide for the curing reaction. Treatment 1 was significantly lower in residual nitrate ($P<0.05$) when compared to all other treatments except for the negative control (C-). Treatments 5 and 6 were not significantly different in residual nitrate levels when compared to the nitrate added control (C+).

Figure 8. Least squares means differences in residual nitrate^a of uncured, no-nitrate-or-nitrite-added (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.



^aResidual nitrate values reported in ppm.

^bTreatments (TRT): 1-4 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages and C+ = nitrite added control and C- = uncured control.

^cSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added and nitrite added EFSC sausages.

^{d-j} Means with different superscripts are different (P<0.05).

Residual Nitrite

Residual nitrite analysis was determined at stuffing, post-incubation (depending upon treatment) and throughout the 90-day storage period. The nitrite added control (C+) was significantly higher in residual nitrite at stuffing when compared to all other treatments ($P < 0.05$) except TRTs 4 and 6. The cherry powder present in the TRT 5 antimicrobial may have caused the significantly lower residual nitrite level when compared to the nitrite added control, as ascorbic acid has a higher reactivity than the erythorbate that was used in the nitrite added control, which would have accounted for the decrease in residual nitrite concentration. Significant differences existed between TRTs 1, 2, and 3 in residual nitrite concentration post incubation and values ranged from 5.49ppm to 38.10ppm. These results further explain the lack of nitrate conversion that took place during the incubation period.

Significant ($P < 0.05$) interactions of treatment*day for treatment combinations were present for residual nitrite and are found in Table 2. Residual nitrite levels decreased overtime for all treatments as expected. Storage temperature was held constant and would not be an underlying factor in the decrease in residual nitrite, however, the effects of increased storage time and packaging conditions (Hustad and others 1973; Ahn and others 2002) has been observed. In general, as storage time increases residual nitrite decreases and the reducing conditions present in the

Table 2. Least squares means for interaction of treatment*day for residual nitrite (ppm) of uncured, no-nitrate-or-nitrite-added (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.

TRT ^a	Day				
	0	14	28	56	90
1	41.92 ^f	31.61 ^e	28.27 ^e	22.74 ^e	17.47 ^{deg}
2	21.23 ^c	14.04 ^c	11.67 ^c	9.98 ^{bc}	9.35 ^{bc}
3	9.24 ^b	6.67 ^b	6.73 ^b	5.97 ^{ab}	7.44 ^{ab}
4	41.86 ^f	32.82 ^e	30.39 ^e	24.85 ^e	20.22 ^{fg}
5	30.27 ^d	22.36 ^d	21.15 ^d	15.40 ^d	13.62 ^{ce}
6	36.31 ^e	29.55 ^e	28.09 ^e	22.15 ^e	17.77 ^{ef}
C+	46.31 ^f	33.28 ^e	23.37 ^e	13.90 ^c	12.73 ^{cd}
C-	2.20 ^a	1.37 ^a	1.68 ^a	1.79 ^a	3.91 ^a
SEM^b	2.39	2.39	2.39	2.39	2.39

^aTreatments (TRT): 1-4 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages and C+ = nitrite added control and C- + uncured control.

^bSEM = Standard error of the means for uncured, no-nitrate-or-nitrite-added (TRTs 1-6) and uncured (C-) and nitrite added (C+) controls.

^{c-e}Means within column with different superscripts are different (P<0.05).

vacuum packaged system would provide reducing conditions and consequently the conversion of residual nitrite to nitric oxide.

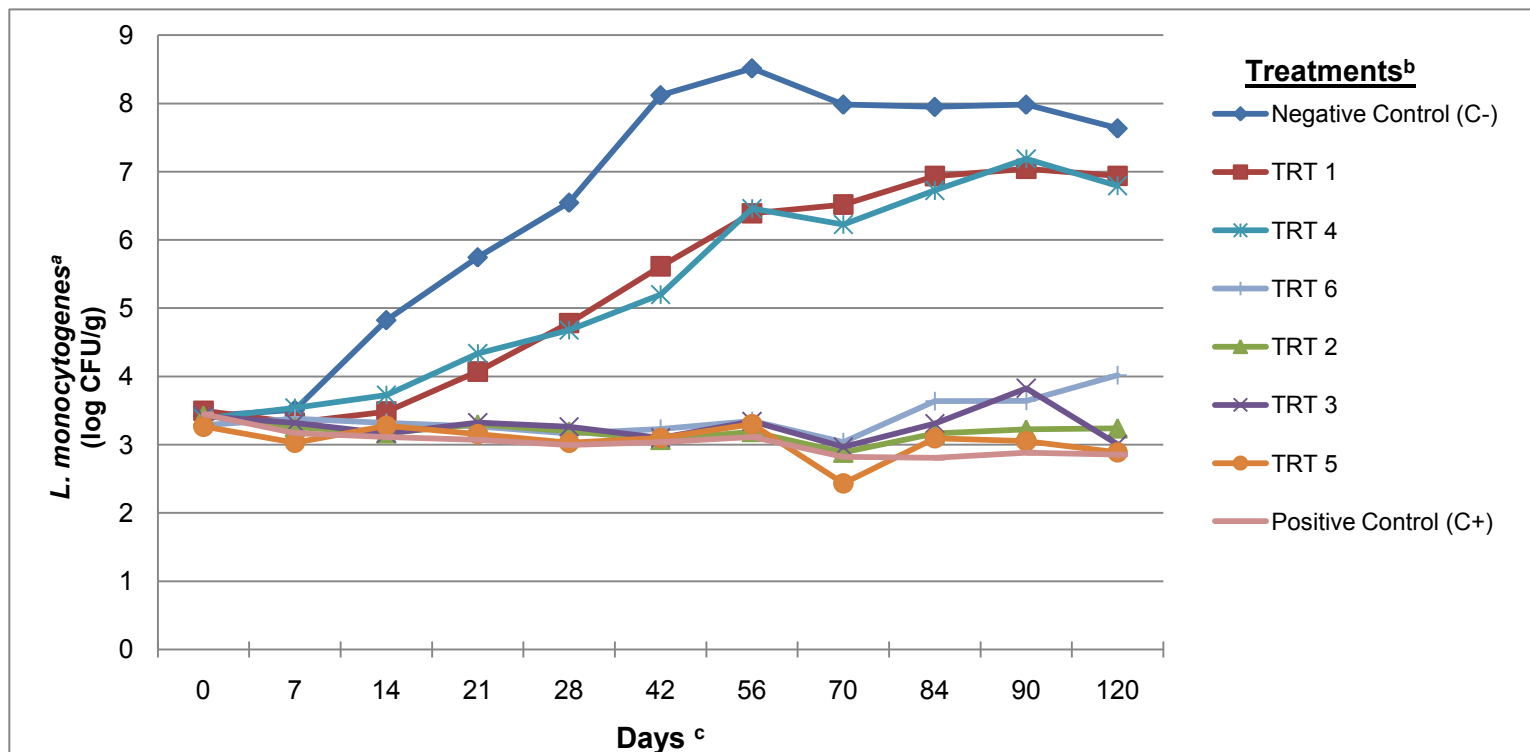
Residual nitrite levels were negatively correlated to TBARS, and positively correlated to a^* values ($P < 0.05$). A trend was observed ($P < 0.10$) for a negative correlation between residual nitrate and residual nitrite. Most importantly, nitrite was found to have a significant negative correlation ($P < 0.05$) between log growth of *L. monocytogenes*, indicating that higher levels of residual nitrite would result in lower levels of microbial growth.

Growth of Listeria monocytogenes

Significant differences ($P < 0.05$) existed between treatments and the overall growth of *L. monocytogenes*. As expected, the negative control (C-) was significantly higher in overall growth over all other treatments ($P < 0.05$). Treatments 1 and 4 were not significantly different from each other ($P > 0.05$) and all uncured no-nitrate-or-nitrite-added treatments which contained natural or clean label antimicrobials were not significantly different from the nitrite-added control, and showed little to no growth throughout the challenge study. The growth of all treatments over time is outlined in Figure 9. Furthermore, all quality attributes which were correlated to the growth of *L. monocytogenes* are outlined in Table 3.

A comparison of antimicrobials as outlined in Table 4, demonstrates no significant difference between the log growths of *L. monocytogenes* in uncured, no-nitrate-or-nitrite-added treatments which contained either antimicrobial when

Figure 9. Growth of *L. monocytogenes*^a on uncured, no-nitrate-or-nitrite-added (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.



^aGrowth of *L. monocytogenes* reported in log CFU/g.

^bTreatments (TRT): 1-4 = different treatments of uncured no-nitrate-or-nitrite-added EFSC sausages and C+ = nitrite added control and C- = uncured control.

^cDays of storage at 4°C over a 120 day study.

Table 3. Pearson's correlation values^a for comparison of quality factors that impact the growth of *L. monocytogenes* on uncured, no-nitrate-or-nitrite-added and control EFSCS treatments (TRT 1-4) and nitrite added control (C+) uncured control (C-) EFSC sausages.

a_w	pH	NITRITE	NITRATE	SALT
0.26899**	-0.07818	-0.22941**	-0.56012**	-0.02601

^aR-Values reported

** (P<0.05) significantly correlated to growth of *L. monocytogenes*.

Table 4. Comparison of natural and “clean label” antimicrobials on uncured, no-nitrate-or-nitrite-added TRTs (2, 3, 5 and 6) and nitrite added (C+) EFSCS.

	0	14	28	56	90
<i>VegStable</i> TM -Control	0.4586	0.4958	0.6243	0.3621	0.1943
Verdad TM -Control	0.4814	0.4301	0.3681	0.1153	0.0135**
<i>VegStable</i> TM -Verdad TM	0.9628	0.8918	0.6073	0.3821	0.095*

^aR-Values reported

* (P<0.10) trend for differences in growth of *L. monocytogenes*.

** (P<0.05) significantly different in growth of *L. monocytogenes*.

compared to the control through day 56 of storage. On day 90 however, a significantly higher population was observed for TRTs 3 and 6 which contained the clean label antimicrobial when compared to the nitrite added control. Furthermore the TRTs 2 and 5 containing the natural antimicrobial tended ($P < 0.10$) to have a lower level of *L. monocytogenes* present when compared to treatments 3 and 6 which received the clean label antimicrobial.

Conclusions

Uncured, no-nitrate-or-nitrite-added processed meats that are produced with the intention of replacing nitrite are at a greater risk for outgrowth of *Listeria monocytogenes* if contamination occurs. No-nitrate-or-nitrite-added treatments that contained no antimicrobial interventions (TRTs 1, 4) did not repress the growth of *L. monocytogenes* throughout the 120 day sampling period when compared to TRTs 2, 3, 5, and 6 which contained an antimicrobial agent. TRTs 1 and 4 mirrored the growth of the negative control which lacked nitrite and anti-listerial controls and resulted in a final population of *Lm* that was 4-5 log CFU/g greater than that of the nitrite added control and the alternatively cured products with alternative antimicrobials.

The results of this study indicate that alternative antimicrobials have a positive impact on the suppression of *Listeria monocytogenes* throughout storage. Further investigations are needed to evaluate these non-meat ingredients to determine if sensory properties would be adversely affected. Furthermore, the negative impact

of the added antimicrobials on the alternative curing system in which a starter culture and nitrate containing vegetable powder are used is cause for concern. While decreased amount of nitrate conversion and in turn lowered levels of residual nitrite do not appear to have an impact on the growth of *L. monocytogenes* in this study, greater implications may arise if anaerobic spore forming pathogens where nitrite concentration is critical for inhibition were present.

The results of this study further emphasize the need for additional antimicrobial measures for natural and organic, uncured, no-nitrate-or-nitrite-added RTE processed meats. Alternative antimicrobials offer natural and organic label friendly solutions to control *L. monocytogenes* in minimally processed meats through 90 d of storage. In doing so these products allow processors to meet USDA-FSIS regulations and can provide consumers with the level of safety that is expected of conventionally cured meat products.

Acknowledgments

This research was supported through a grant funded by the National Integrated Food Safety Initiative (Grant no. 2006-51110-03609) of the United States Department of Agriculture Cooperative State Research, Education, and Extension Service.

References

- [AOAC] Association of Official Analytical Chemists. 1990. Nitrites in cured meat. Official Method 973.31. Official Methods of Analysis. 15th ed. Arlington, Va: AOAC.
- Ahn DU & Maurer AJ. 1987. Concentration of nitrate and nitrite in raw turkey breast meat and the microbial conversion of added nitrate to nitrite in tumbled turkey breast meat. *Poult Sci* 66:1957-1960.
- Ahn HJ, Kim JH, Jo C, Lee CH & Byun MW. 2002. Reduction of carcinogenic N-nitrosamines and residual nitrite in model system sausage by irradiation. *J Food Sci* 67:1370-1373.
- Bacus JN. 2006. Natural ingredients for cured and smoked meats. Proceedings of the 59th Annual American Meat Science Association Reciprocal Meat Conference. Champaign-Urbana, Illinois. p. 77-78.
- Cassens RG. 1997. Residual nitrite in cured meat. *Food Technol* 51(2):53-55.
- Code of Federal Regulations. 2003. Control of *Listeria monocytogenes* in ready-to eat meat and poultry products; Final Rule. 9CFR 430. Washington, DC: U.S. Government Printing Office.
- Fox JB, Jr. 1966. The chemistry of meat pigments. *J Agri Food Chem* 14:207-210.
- Glass KA & Doyle MP. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl Environ Microbiol* 55(6):1565-1569.
- Hustad GO, Cervený JG, Trenk H, Deibel RH, Kautter DA, Fazio T, Johnston RW & Kolari OE. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in wieners. *Appl Environ Microbiol* 26(1):22-26.
- Jay JM, Loessner MJ & Golden DA. 2005. Modern food microbiology, Seventh ed. New York, NY: Springer Science and Business Media LLC.
- Lianou A, Geornaras I, Kendall PA, Belk KE, Scanga JA, Smith GC & Sofos JN. 2007. Fate of *Listeria monocytogenes* in commercial ham, formulated with or without antimicrobials, under conditions simulating contamination in the processing or retail environment and during home storage. *J Food Prot* 70(2):378-385.

- Maas MR, Glass KA & Doyle MP. 1989. Sodium lactate delays toxin production by *Clostridium botulinum* in cook-in-bag turkey products. *Appl Environ Microbiol* 55(9):2226-2229.
- MacDonald B, Gray JI & Gibbins LN. 1980. Role of nitrite in cured meat flavor: antioxidant role of nitrite. *J Food Sci* 45(4):893-897.
- Price JF & Schweigert BS. 1987. *The science of meat and meat products*, 3rd ed. Westport, CT: Food and Nutrition Press, Inc.
- SAS Institute. 2008. *SAS/STAT 9.2 user's guide*. Cary, N.C.: SAS Institute Inc.
- Sebranek JG & Bacus JN. 2007. Cured meat products without direct addition of nitrate or nitrite: what are the issues? *Meat Sci* 77(1):136-147.
- Sindelar JJ. 2006. Investigating uncured no-nitrate-or-nitrite-added processed meat products. Ph.D. Dissertation. Ames, IA, USA: Iowa State University. 247p
- Sindelar JJ, Cordray JC, Olson DG, Sebranek JG & Love JA. 2007a. Investigating quality attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial hams, bacons, and frankfurters. *J Food Sci* 72(8):551-559.
- Sindelar JJ, Cordray JC, Sebranek JG, Love JA & Ahn DU. 2007b. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. *J Food Sci* 72(6):388-395.
- Tarladgis BG. 1962. Interpretation of the spectra of meat pigments. II. - Cured meats. The mechanism of colour fading. *J Sci Food Agri* 13(9):485-491.
- Tompkin RB. 1995. Nitrite. In: Davidson, P. M., Sofos, J. N. & Brannen, A. L., editors. *Antimicrobials in food*. 3rd ed. Boca Raton, FL: CRC Press, Taylor & Francis Group.

CHAPTER 5. GENERAL CONCLUSIONS

The objective of evaluating commercially available uncured, no-nitrate-or-nitrite-added products for the control of *Listeria monocytogenes* was to determine the level of safety which these products include. As hypothesized, conventional meat products which contained the direct addition of nitrite and antilisterial controls including lactate or diacetate demonstrated superior control of the pathogen when compared to the minimally processed no-nitrate-or-nitrite-added brands. It was demonstrated during these experiments that products which are manufactured with the intention of replacing nitrite lack the ability to suppress the growth of *Listeria monocytogenes*. Interestingly, brands which included the ingredient sodium lactate in the formulation within particular classes of uncured, no-nitrate-or-nitrite-added meat products were able to increase the lag phase and generation time for initiation of growth when compared to products which did not contain lactate at reduced levels. Nevertheless, significant differences existed between conventionally processed meat products and their minimally processed counterparts establishing that an increased risk for the deadly foodborne pathogen *L. monocytogenes* existed. Fears of such a hazard prompted evaluation of commercially available natural and “clean label” antimicrobials for improved safety of these products.

Natural blends of cherry powder, lemon powder and vinegar as well as label friendly solutions which contained cultured corn sugar and vinegar dramatically improved the ability of minimally processed emulsified frankfurter style cooked sausages to suppress the growth of *L. monocytogenes* throughout 120 days of

storage. While these products were not significantly different from conventionally processed controls which contained the direct addition of nitrite and antilisterial measures, they did have negative implications on natural curing systems. *Staphylococcus carnosus* starter cultures utilized in alternative curing systems for the production of nitrite were unable to convert the nitrate containing source within the incubation period when natural and clean label antimicrobials were added. Significant differences existed in residual nitrate and nitrite post incubation, indicating that the added anti-listerial control measures reduced the nitrite reductase activity of the starter culture. Further investigations would determine if additional time is necessary for the incubation period and if the dramatically reduced levels of ingoing nitrite that result from the use of added antimicrobials have implications on the control of pathogenic sporeforming bacteria.

APPENDICIES

LIST OF APPENDICIES

- APPENDIX 1: Product brand designations and product ingredient statements for commercial uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) frankfurters used in study
- APPENDIX 2: Product brand designations and product ingredient statements for commercial uncured, no-nitrate-or-nitrite-added (Brand D-H) and nitrite added (Brand A-C) deli hams used in study
- APPENDIX 3: Uncured, No-Nitrate-Nitrite-Added EFSC Sausage Experiment Formulations
- APPENDIX 4: CHR Hansen CS 299 Bactoferm™ Product Information
- APPENDIX 5: Florida Food Products VegStable™ 502 Product Information
- APPENDIX 6: Florida Food Products VegStable™ 504 Product Information
- APPENDIX 7: Florida Food Products VegStable™ 507 Product Information
- APPENDIX 8: Purac PuraQ™ Verdad™ NV 55 Product Information

APPENDIX 1: Product brand designations and product ingredient statements for commercial uncured, no-nitrate-or-nitrite-added (Brand C-J) and nitrite added (Brand A-B) frankfurters used in study

CODE	BRAND	INGREDIENT STATEMENT
A	BALL PARK	INGREDIENTS: BEEF, WATER, CORN SYRUP, CONTAINS 2% OR LESS OF: SALT, POTASSIUM LACTATE, PARTIALLY HYDROLYZED BEEF STOCK, SODIUM PHOSPHATES, FLAVORINGS, SODIUM DIACETATE, ASCORBIC ACID (VITAMIN C), SODIUM NITRITE, EXTRACTIVES OF PAPRIKA.
B	OSCAR MAYER	INGREDIENTS: BEEF, WATER, CONTAINS LESS THAN 2% OF SALT, CORN SYRUP, DEXTROSE, SODIUM LACTATE, SODIUM PHOSPHATES, SODIUM DIACETATE, ASCORBIC ACID (VITAMIN C), EXTRACTIVES OF PAPRIKA, DRIED GARLIC, SPICE, SODIUM NITRITE.
C	NIMAN RANCH – FEARLESS FRANKS	INGREDIENTS: BEEF, WATER, SALT, DEXTROSE, MUSTARD, PAPRIKA, GARLIC, SODIUM PHOSPHATE, CELERY JUICE POWDER, NONFAT DRY MILK, LACTIC ACID STARTER CULTURE, SPICES.
D	WELLSHIRE FARMS	INGREDIENTS: BEEF, WATER, SEA SALT, EVAPORATED CANE JUICE, CELERY POWDER, NATURAL FLAVORINGS.
E	APPLEGATE FARMS – UNCURED ORGANIC	INGREDIENTS: ORGANIC BEEF, WATER. CONTAINS LESS THAN 2% OF THE FOLLOWING: SEA SALT, ORGANIC CELERY JUICE, SODIUM LACTATE (FROM BEETS), LACTIC ACID STARTER CULTURE (NOT FROM MILK), ORGANIC ONION POWDER, ORGANIC SPICES, ORGANIC PAPRIKA.
F	ORGANIC PRAIRIE	INGREDIENTS: ORGANIC BEEF, WATER, SODIUM LACTATE, SEA SALT, ORGANIC TURBINADO SUGAR, NATURAL FLAVOR, ORGANIC SPICES, ORGANIC ONION POWDER, ORGANIC GARLIC POWDER, LACTIC ACID STARTER CULTURE.
G	OSCAR MAYER - NATURAL	INGREDIENTS: BEEF, WATER, CONTAINS LESS THAN 2% OF SEA SALT, POTASSIUM LACTATE (FROM CORN), EVAPORATED CANE JUICE, CELERY POWDER, FLAVOR, LACTIC ACID STARTER CULTURE.
H	APPLEGATE FARMS – GREAT UNCURED ORGANIC	INGREDIENTS: ORGANIC GRASS-FED BEEF, WATER, CONTAINS LESS THAN 2% OF THE FOLLOWING: SEA SALT, ORGANIC SPICES, ORGANIC GARLIC POWDER, ORGANIC PAPRIKA, CELERY JUICE, ORGANIC ONION, LACTIC ACID STARTER CULTURE (NOT FROM MILK).
I	WHOLESOME HARVEST OLD FASHION FRANKS	INGREDIENTS: BEEF, MECHANICALLY SEPARATED CHICKEN, WATER, SALT, SUGAR, SPICES.
J	WHOLE RANCH	INGREDIENTS: BEEF, WATER, SEA SALT, HONEY, SPICES, ONION, GARLIC.

APPENDIX 2: Product brand designations and product ingredient statements for commercial uncured, no-nitrate-or-nitrite-added (Brand D-H) and nitrite added (Brand A-C) deli hams used in study

CODE	BRAND	INGREDIENT STATEMENT
A	HORMEL CURE 81	CURED WITH: WATER, SALT, POTASSIUM LACTATE, DEXTROSE, MODIFIED POTATO STARCH, PHOSPHATE, ERYTHORBATE, SODIUM NITRITE AND SODIUM DIACETATE.
B	JOHN MORRELL	CURED WITH: WATER, DEXTROSE, CONTAINS 2% OR LESS OF SALT, POTASSIUM LACTATE, SODIUM LACTATE, SODIUM PHOSPHATE, SODIUM DIACETATE, SODIUM ERYTHORBATE AND SODIUM NITRITE.
C	FARMLAND	CURED WITH: WATER, DEXTROSE, SALT, POTASSIUM LACTATE, SODIUM PHOSPHATES, SODIUM DIACETATE, SODIUM ERYTHORBATE AND SODIUM NITRITE.
D	OSCAR MAYER NATURAL HAM	INGREDIENTS: PORK WATER, CONTAINS LESS THAN 2% OF POTASSIUM LACTATE (FROM CORN), SEA SALT, EVAPORATED CANE JUICE, CELERY POWDER, AND LACTIC ACID STARTER CULTURE.
E	WELLSHIRE BLACK FORREST HAM	INGREDIENTS: PORK, WATER, SUGAR, SEA SALT, NATURAL FLAVORING, MAPLE SUGAR, AND DRIED HONEY.
F	BEELER'S UNCURED HAM	INGREDIENTS: PORK WATER, SEA SALT, TURBINADO SUGAR, AND CELERY POWDER.
G	APPLEGATE FARMS ORGANIC UNCURED HAM	INGREDIENTS: ORGANIC PORK, WATER, CONTAINS LESS THAN 2% OF THE FOLLOWING: SEA SALT, SODIUM LACTATE (FROM BEETS), ORGANIC HONEY, ORGANIC SUGAR, CELERY POWDER, AND LACTIC ACID STARTER CULTURE.
H	HORMEL NATURAL CHOICE™ SMOKED DELI HAM	INGREDIENTS: WATER, SALT, TURBINADO SUGAR, NATURAL FLAVOR, AND LACTIC ACID STARTER CULTURE (NOT FROM MILK).

APPENDIX 3: Uncured, no-nitrate-nitrite-added EFSC Sausage Experiment Formulations

Control: Uncured(Negative Control)

Meat Block:

Beef Trim – 80/20	15.0 lbs
Pork Trim – 50/50 (60% Chemical Lean)	15.0 lbs

Ingredients:

Water/Ice (20%)	6.0 lbs	
A.C. Legg Spice Pack	243.23 gm	
Salt (2.25%)	0.675 lbs	306.17 gm
Dextrose (2.0%)	0.60 lbs	272.15 gm

Control: Nitrite Added (Positive Control)

Meat Block:

Beef Trim – 80/20	15.0 lbs
Pork Trim – 50/50 (60% Chemical Lean)	15.0 lbs

Ingredients:

Water/Ice (20%)	6.0 lbs	
A.C. Legg Spice Pack	243.23 gm	
Salt (2.25%)	0.675 lbs	306.17 gm
Dextrose (2.0%)	0.60 lbs	272.15 gm
Sodium Nitrite (6.25% Cure)	0.075 lbs	33.96 gm
Sodium Erythorbate	0.0165 lbs	7.494 gm
Optiform PD.4 (2.5%)	0.75 lbs	340.5 gm

Treatment 1: VegStable 502/CS-299 Starter Culture**Meat Block:**

Beef Trim – 80/20	15.0 lbs
Pork Trim – 50/50 (60% Chemical Lean)	15.0 lbs

Ingredients:

Water/Ice (20%)	6.0 lbs	
A.C. Legg Spice Pack	243.23 gm	
Salt (2.25%)	0.675 lbs	306.17 gm
Dextrose (2.0%)	0.60 lbs	272.15 gm
VegStable 502	0.0756 lbs	34.332 gm
(0.20% of total formulation)		
Starter Culture (CS-299)	4.41 gm	
(35 gm/300 lb Meat)		

Treatment 2: VegStable 502/CS-299 Starter Culture/VegStable 507 – CPlus**Meat Block:**

Beef Trim – 80/20	15.0 lbs
Pork Trim – 50/50 (60% Chemical Lean)	15.0 lbs

Ingredients:

Water/Ice (20%)	6.0 lbs	
A.C. Legg Spice Pack	243.23 gm	
Salt (2.25%)	0.675 lbs	306.17 gm
Dextrose (2.0%)	0.60 lbs	272.15 gm
VegStable 502 (0.2%)	0.0756 lbs	34.332 gm
Starter Culture (CS-299)	4.41 gm	
(35 gm/300 lb Meat)		
VegStable 507 (1.4% total formulation)	0.5305 lbs	240.87 gm

Treatment 3: VegStable 502/CS-299 Starter Culture/Verdad 55**Meat Block:**

Beef Trim – 80/20	15.0 lbs
Pork Trim – 50/50 (60% Chemical Lean)	15.0 lbs

Ingredients:

Water/Ice (20%)	6.0 lbs	
A.C. Legg Spice Pack	243.23 gm	
Salt (2.25%)	0.675 lbs	306.17 gm
Dextrose (2.0%)	0.60 lbs	272.15 gm
VegStable 502 (0.2%)	0.0756 lbs	34.332 gm
Starter Culture (CS-299)	4.41 gm	
(35 gm/300 lb Meat)		
Verdad 55 (3%)	0.9 lbs	408.6 gm

Treatment 4: VegStable 504**Meat Block:**

Beef Trim – 80/20	15.0 lbs
Pork Trim – 50/50 (60% Chemical Lean)	15.0 lbs

Ingredients:

Water/Ice (20%)	6.0 lbs	
A.C. Legg Spice Pack	243.23 gm	
Salt (2.25%)	0.675 lbs	306.17 gm
Dextrose (2.0%)	0.60 lbs	272.15 gm
VegStable 504	0.1701 lbs	77.24 gm
(0.45% of total formulation)		

Treatment 5: VegStable 504/VegStable 507 – CPlus**Meat Block:**

Beef Trim – 80/20	15.0 lbs
Pork Trim – 50/50 (60% Chemical Lean)	15.0 lbs

Ingredients:

Water/Ice (20%)	6.0 lbs	
A.C. Legg Spice Pack	243.23 gm	
Salt (2.25%)	0.675 lbs	306.17 gm
Dextrose (2.0%)	0.60 lbs	272.15 gm
VegStable 504	0.1701 lbs	77.24 gm
(0.45% of the total product formulation)		
VegStable 507	0.5317 lbs	241.41 gm

Treatment 6: VegStable 504/Verdad 55**Meat Block:**

Beef Trim – 80/20	15.0 lbs
Pork Trim – 50/50 (60% Chemical Lean)	15.0 lbs

Ingredients:

Water/Ice (20%)	6.0 lbs	
A.C. Legg Spice Pack	243.23 gm	
Salt (2.25%)	0.675 lbs	306.17 gm
Dextrose (2.0%)	0.60 lbs	272.15 gm
VegStable 504	0.1701 lbs	77.24 gm
(0.45% of the total product formulation)		
Verdad 55 (3%)	0.9 lbs	408.6 gm

Processing Schedule:

1. Grind lean (beef) and fat trimmings (pork) through ½" plate.
2. Place lean trimmings, salt, ½ of water/ice and vegetable powder in the bowl chopper.
3. Add the rest of the dry ingredients into chopper and chop to 36°F.
4. Add 50/50 pork trim and remaining water/ice and chop to 55-59°F.
5. Stuff into impermeable plastic casing.
6. Split treatments into two trucks for appropriate thermal processing procedures. Incubation time begins when internal temperature of product reaches 100°F.
7. Thermal Process to internal temperature of 160°F.
8. Place in cooler and chill to below 40°F.

Smokehouse Schedule for Curing Systems Requiring Incubation (TRT 1, 2, 3):

Step	Time	Dry Bulb (°F)	Wet Bulb (°F)	Relative Humidity	Dampers
1 (Cook)	IT=100	105	103	93%	Auto
2 (Cook)	60 min	105	103	93%	Auto
3 (Cook)	20 min	140	110	38%	Auto
4 (Cook)	20 min	160	145	67%	Auto
5 (Cook)	IT=160	185	178	85%	Auto
6 (Shower)	15 min				

Smokehouse Schedule for Curing Systems Requiring No Incubation Period (TRT 4, 5, 6) and Controls (Nitrite Added and Uncured):

Step	Time	Dry Bulb (°F)	Wet Bulb (°F)	Relative Humidity	Dampers
1 (Cook)	20 min	140	110	38%	Auto
2 (Cook)	20 min	160	145	67%	Auto
3 (Cook)	IT=160	185	178	85%	Auto
4 (Shower)	15 min				

APPENDIX 4: CHR Hansen CS 299 Bactoform™ Product Information

CHR HANSEN

CS 299 Bactoform™

Product information

Description	<p>Meat culture for enhancing color and flavor formation in cured, spreadable and fermented meat products acidified by natural or chemical means. This culture is a meat starter cultures producing lactic acid and other flavor compounds.</p> <p>CS 299 is a highly concentrated single strain culture containing <i>Staphylococcus carnosus</i> in a convenient freeze-dried form. <i>S. carnosus</i> enhances the formation of a pleasant mild flavor profile and a good stable color.</p>	
Application	<p>CS 299 is recommended in the production of all fermented cured sausages, sliceable as well as spreadable, in which the acidification is obtained by means of lactic acid bacteria or chemical acidulants (GDL, citric acid or likewise).</p>	
Packing	<p>Packing size</p> <p>25g for 225kg</p>	<p>Material number</p> <p>673911</p>
Storage and shelf life	<p>Freeze-dried cultures should be stored at below -17°C (0°F) to have a shelf life for at least 18 months. At +5°C (41°F) the shelf life is at least 6 weeks.</p>	
Instructions for use	<p>Addition to sausage mince: The contents of the pouch should be added directly to the bowl/chopper early in the process together with the dry ingredients.</p> <p>According to USDA regulations/Flavoring Agents this product is labeled as “Lactic Acid Starter Culture”.</p>	
Dosage	<p>Follow the information on the pouch.</p>	
Production temperature	<p>Recommended production temperature is 20°C (68°F) as a minimum in order to ensure activity. The fermentation and drying procedures should be adapted to the specific recipes.</p>	

CS 299 PI_EN_vs4 Feb 05.doc 1:2

CS 299 Bactoform™

Product Information



Technical data

Culture	CS 299
Bacteria strain composition	<i>Staphylococcus carnosus</i>
Temperature	
Optimum/maximum/minimum	30°C/45°C/10°C (86°F/113°F/50°F)
Salt limit	16% salt-in-water
Characteristics	Facultative anaerobic Catalase positive Nitrate reductase positive Lipolytic Proteolytic DL(+/-) lactic acid producing
Fermentable sugars	
Glucose (dextrose)	+
Fructose	+
Maltose	-
Lactose	+
Saccharose (sucrose)	-
Starch	-
Carrier	Glucose (dextrose)
Appearance	White powder with brownish particles
Packing	Freeze-dried culture packed in water and air proof aluminum foil pouch

Technical service

Chr. Hansen's worldwide facilities and the personnel of our Application and Technology Centers are at your disposal with assistance, instructions and guidance for your choice of culture and needs for spice and seasoning blends.

References

References and analytical methods are available upon request.

The information contained herein is to our knowledge true and correct and presented in good faith. However, no warranty, guarantee, or freedom from patent infringement is implied or inferred. This information is offered solely for your consideration and verification.

CS 299 PI_EN_vs4 Feb 05.doc 2:2

APPENDIX 5: Florida Food Products VegStable™ 502 Product Information



FLORIDA FOOD PRODUCTS, INC.
2231 W. Hwy 44 • Post Office Box 1300 • Eustis, Florida USA 32727-1300
352/357-4141 • Fax 352/483-3192 • E-Mail: contact@floridafood.com

PRODUCT NAME – Veg Stable™ 502

INGREDIENT DECLARATION – Celery powder (or natural flavors), sea salt.

USE – Meats, dry soups and seasoning blends.

DESCRIPTION – Veg Stable™ 502 is a water-soluble dried powder consisting of celery powder and sea salt. Veg Stable™ 502 is high in naturally occurring nitrates that are standardized with sea salt. Anti-caking agents may be added.

USE RATE - 0.1%-0.2%

GENERAL SPECIFICATIONS

Veg Stable™ 502

APPEARANCE	Tan to brown, free flowing powder
MOISTURE	≤ 5%
pH (5% solution)	6.0 - 7.0
TOTAL PLATE COUNT	≤ 20,000 cfu/gm
YEAST & MOLD	≤ 100 cfu/gm
TOTAL COLIFORMS	Negative
PRESERVATIVES	None

PACKAGING - Available in 44.1 lb (20 kg) vacuum-sealed foil bag-n-box.

SHELF STABILITY AND STORAGE CONDITIONS - Store in a cool and dry area not exceeding 70°F. When properly stored vacuum sealed, the recommended shelf life is one

AVAILABILITY – Veg Stable™ 502 is available year round from inventory. Advance notice for quantities above 2,200 lbs is required.

PROCESSING SEASON - December - June

The technical information and suggestions for use contained herein are believed to be reliable, but they are not to be construed as warranties and no patent liability can be assumed. Specifications are subject to change based on raw material variations.

Revised: 6/5/08 *Growers and Processors of Food and Cosmetic Ingredients*
Supersedes: 5/22/08 Visit our Web Site at: www.floridafood.com

SP502

APPENDIX 6: Florida Food Products VegStable™ 504 Product Information



FLORIDA FOOD PRODUCTS, INC.
 2231 W. Hwy 44 • Post Office Box 1300 • Eustis, Florida USA 32727-1300
 352/357-4141 • Fax 352/483-3192 • E-Mail: contact@floridafood.com

PRODUCT NAME – Veg Stable™ 504

INGREDIENT DECLARATION – Celery powder (or natural flavors), sea salt.

USE – Meats, natural curing processes

DESCRIPTION – Veg Stable™ 504 is a water-soluble dried powder consisting of celery powder and sea salt. Veg Stable™ 504 is high in naturally occurring nitrites that are standardized with sea salt. Anti-caking (silicon dioxide) agents may be added.

<u>GENERAL SPECIFICATIONS</u>	<u>Veg Stable™ 504</u>
-------------------------------	------------------------

APPEARANCE	Tan to Brown free flowing powder
MOISTURE	≤5%
pH (5% solution)	8.5 - 10
TOTAL PLATE COUNT	20,000 cfu/gm
YEAST & MOLD	100 cfu/gm max.
TOTAL COLIFORMS	Negative
PRESERVATIVES	None

PACKAGING - Available in 44.1 lb. vacuum-sealed foil bag-n-box.

SHELF STABILITY AND STORAGE CONDITIONS - Store in a cool and dry area not exceeding 70°F. When properly stored vacuum sealed, the recommended shelf life is one year.

SUGGESTED USAGE - to 0.2 - 0.4% of gross weight

AVAILABILITY – Veg Stable™ 504 is available year round from inventory. Advance notice for quantities above 2,200 lbs. is required.

PROCESSING SEASON - December - June

The technical information and suggestions for use contained herein are believed to be reliable, but they are not to be construed as warranties and no patent liability can be assumed. Specifications are subject to change based on raw material variations.

APPENDIX 7: Florida Food Products VegStable™ 507 Product Information



FLORIDA FOOD PRODUCTS, INC. Product Specifications and Information

PRODUCT NAME – VEG STABLE™ C-PLUS #507

Code # 507

INDEPENDENT DECLARATION –Vinegar, Lemon Powder, Cherry Powder

DESCRIPTION – Veg Stable™ C-PLUS is an all natural blend of vinegar and fruit powders designed for improved shelf stability in meat systems.

USE RATE - .12% -1.4%

GENERAL

APPEARANCE

MOISTURE

Ph (3% solution)

TOTAL PLATE COUNT

YEAST & MOLD

TOTAL COLIFORMS

PRESERVATIVES

Veg Stable™ CPLUS

Yellow/Tan free flowing powder

<5%

5.5 – 6.2

3,000 cfu/gm max.

100 cfu/gm max.

Negative

None

PACKAGING – Available in 44.1 lb. vacuum-sealed foil bag-n-box.

SHELF STABILITY AND STORAGE CONDITIONS – Store in a cool and dry area not exceeding 70°F. When properly stored vacuum sealed, the recommended shelf life is one year.

AVAILABILITY – Veg Stable™ CPLUS is available year round from inventory. Advance notice for quantities above 5,000 lbs. is required.

PROCESSING SEASON – December – June

The technical information and suggestions for use contained herein are believed to be reliable, but they are not to be construed as warranties and no patent liability can be assumed. Specifications are subject to change based on raw material variations.

SP507.doc

APPENDIX 8: Purac PuraQ™ Verdad™ NV 55 Product Information



Product data

Rev No.5/4511

PuraQ™ Verdad™ NV55

PuraQ Verdad NV55 is a natural product based on two label friendly ingredients: cultured sugar and vinegar. Cultured sugar is produced by fermentation with specifically selected food cultures. This unique product consists of fermentation products such as sugars, organic acids, peptides and/or aromas.

Our cultured products are developed to impart the benefits of fermentation without having to ferment the food product. PuraQ Verdad NV55 is specifically designed to apply in uncured meat & poultry products.

Specification	Product Form	cultured sugar & vinegar liquid
Assay	Organic acid salts (meq per 100 g product, titration)	490-520
	Dry matter	59-65% w/w
	Assay vinegar	3-6% w/w
	Assay sodium	max. 5%
	Sugar	max. 1%
Visual sensoric characteristics	Color (Gardner)	3-18
Purity	pH (direct)	6-9
	pH (10%)	5-8
Registration	FDA	cultured sugar – self affirmed GRAS
	USDA/FSIS	vinegar – GRAS
	CAS number	Directive 7120.1
	OSHA 1910.1200/EU regulation	8028-52-2 (vinegar)
		non hazardous
Labeling	USDA	cultured sugar, vinegar
Intended Use	Meat and poultry products	

Copyright © Purac. All rights reserved. No part of this publication may be copied, downloaded, reproduced, stored in a retrieval system or transmitted in any form by any means, electronic, mechanical, photocopied, recorded or otherwise, without permission of the publisher. No representation or warranty is made as to the truth or accuracy of any data, information or opinions contained herein or as to their suitability for any purpose, condition or application. None of the data, information or opinions contained herein may be relied upon for any purpose or reason. Purac disclaims any liability, damages, losses or other consequences suffered or incurred in connection with the use of the data, information or opinions contained herein. In addition, nothing contained herein shall be construed as a recommendation to use any products in conflict with existing patents covering any material or its use.

ACKNOWLEDGEMENTS

“Most people can look back over the years and identify a time and place at which their lives changed significantly. Whether by accident or design, these are the moments when, we make certain choices that will affect the rest of our lives.”

– Frederick F. Flack

No quote could better summarize how I feel about moving to Ames, Iowa and pursuing not only a Bachelors degree in Animal Science, but a Doctoral degree in Meat Science. I am proud to be a Cyclone and both honored and humbled to have received my education from the leading Meat Science program in the country. For those who truly know me, where I come from and all that I have overcome to be where I am at today, can appreciate what both Iowa State University and the Meat Science program have done for me. I have finally found my passion in life and that is something that may often be imitated but never duplicated. I love who I have become, I love what I do, and it is all because of my experiences at ISU.

It is hard to imagine where I would be without the many influential people that have come in my life since I first walked into 194 Meat Laboratory in August of 2003 looking for a part time job during my undergraduate studies. Now, after nearly 7 years and 2 degrees, my collegiate career at Iowa State University is drawing to a close. There were many days where I was exhausted, overwhelmed and frustrated; thinking that this day would never come. Now that it is here I am excited to see what lies ahead and yet saddened to leave the family that I have come to know and love. So, as Dr. Seuss put it... *“Don’t cry because it is over, smile because it happened.”*

I am not sure how to begin to express the gratitude that I have for the many opportunities that have been presented to me during my time here at Iowa State. I would first like to extend my sincere appreciation to my major professor, mentor and friend, Dr. Joe Cordray. You have further cemented my interest in meat science, and allowed me to grow and become the person I am today. My knowledge of meat science and the meat industry would not be the same without all of your guidance, support, and friendship. Thank you for giving me the chance to travel throughout the country and world to present on various topics about meat science, work with well respected industry professionals and expand on my knowledge so that I can better serve the industry upon my departure from Iowa State. You provided real world learning experiences in the field of meat science and were never too busy to give me guidance and advice when I needed it most. I look forward to returning to Iowa State and assisting you with extension programs in any capacity that I can.

I would also like to extend my appreciation to Dr. Joe Sebranek for not only serving on my graduate committee but for his assistance and support with my research project. I was always envious of Dr. Joe and his ability to write and think like a scientist....and he brought the researcher out in me. Your support throughout my undergraduate and graduate studies was much appreciated. I knew that if you had faith in my abilities...than I should too. Of course the encouragement from my other committee members was no different. I would like to thank Dr. Dennis Olson, Dr. Ken Prusa and Dr. Aubrey Mendonca for their patience, kind words and leadership throughout my time here at Iowa State. I have enjoyed having the

opportunity to work with you all in one capacity or another, and hope that we can continue to work together in years to come.

To my “Meat Lab Family”....I can’t begin to thank you enough for everything you have all done for me both professionally and personally. To Deb Michel, my Meat Lab Mom, all of the Thank You’s in the world would never be enough for all that you have done for me. I have enjoyed working with you over the past several years and value our friendship dearly...I will always be one of “Deb’s Dingers”☺. A big thanks to Randy Petersohn for all of his assistance with not only my research project but all of our short courses as well. It has been a fun ride...from covering your office with packing peanuts, filling it with black balloons on your birthday, bubble wrapping your desk, chair and computer, and throwing you a warm sunny sendoff for your retirement. I will always remember your big hearty laugh and your famous three little words “very good then.” I would also like to extend my gratitude to Jeff Mitchell, Mike Holtzbauer, Steve Bryant and Vail Olson. It has been great having the opportunity to work with you all, and I want you to know that all of your efforts have not gone unnoticed...I couldn’t have done this without each and every one of you. I would also like to thank the Meat Lab undergraduate employees. I was great at making a mess anytime I made product...and I know how fun it was to clean up. I look forward to returning to the meat lab (making a mess) and seeing you all again...I’ll be there for coffee, and I’ll try and remember to make some banana bread for break.

Thanks to Steve Neibuhr in the Food Safety Research Lab. I came in a sausage maker and you turned me into a microbiologist. Thank you for all of your assistance with the pathogen challenge portion of my project and answering the countless questions that I had on sampling techniques and protocols and being patient with my schedule outside of the FSRL lab. I would also like to thank Marcia King-Brink and Elaine Larson for their assistance with various analytical portions of my project...your help was much appreciated! To Dr. Ahn and Dr. Lee's lab, thank you for running the residual nitrate analysis for my project, it was great working with you all.

I would also like to thank the graduate students that I have had the opportunity to work with over the past few years. I would especially like to thank Dr. Jeff Sindelar for his friendship, guidance and support not only while he was here at Iowa State, but after his graduation as well. I would also like to thank Nicolas Lavieri for his assistance in various aspects of my project. It didn't matter if it was in the micro lab or manufacturing product, you were always willing to help in whatever capacity you could. To my fellow officemates in 2371....thank you for putting up with me and making me smile....even when I didn't want to. A big shout out goes to Mark Anderson, for his statistical guidance....I will always have a love/hate relationship with SAS, but I couldn't have performed my analysis or understood the hundreds of pages of print outs without your help. I would also like to thank Sherry Olsen....better known as "Aunt Sherry" to us all. Thank you for your friendship and

always being willing to lend your ear and comfort me with those magic words “it’ll be ok”.

To my parents, Arden and Lisa, thank you for instilling in me the importance of hard work and determination, I would not be where I am today without it. If you were to ask my mother or father, I was destined to be in the meat industry....how many four year old girls are content sitting at a butcher block in their kitchen playing in a bowl of chicken gizzards? I am sure the answer would be few and far between; of course I never have seemed to do it like anybody else, and I am sure that my judgment in “taking the long way around” often worried you both but your love and support never swayed. Thank you for your unconditional love and support throughout my education. To my brother, Kitrick, I am not sure where to begin....you have become one of my closest friends and allies and have always had my back. Thank you for lending me your ear, and letting me vent about my trials and tribulations. You have walked a mile in my shoes and then some, and it was always comforting to know that someone could relate. To my Grandma and Grandpa Krider, thank you for your love, support and friendship. I feel very blessed to have a great support system back home in Indiana....I love you all dearly.

And last...but certainly not least, I would like to thank my rock, my love, my best friend Dr. Jay Wenter. I don’t know how I would have gotten through these last 3 years without your love and support. I look forward to the future and what it has in store for us, the best is truly yet to come.

Finally, to all the future graduate students that may read this...I leave you with this: Enjoy your graduate experience at Iowa State, it is truly second to none. But remember, your experiences here and opportunities after graduation are dependent upon your drive, motivation and determination to be the best that you can be in your chosen field of endeavor. So don't sit in your office, or stay in the lab pipetting all day long. Get downstairs and really understand the process behind all aspects of meat science....slaughter some hogs, skin a beef, fab out a carcass and know how to make a frankfurter...not just by reading it in a book, but by doing it with your own bare hands. Be proactive and above all else passionate about the great industry you are in.